INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 95/04575

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/57 C12N9/64 A61K38/48 G01N33/50 C12Q1/68
C12N5/10 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61K G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	CELL, APR 7 1995, 81 (1) P27-40, UNITED STATES, XP002010548 RICHARD I ET AL: "Mutations in the proteolytic enzyme calpain 3 cause limb-girdle muscular dystrophy type 2A." see the whole document ---	1-20
X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 264, no. 33, 1989, BALTIMORE, MD US, pages 20106-20111, XP002010549 HIROYUKI SORIMACHI ET AL.: "Molecular cloning of a novel mammalian calcium-dependent protease distinct form both m- and mu-types" cited in the application see figures 2,3 --- -/--	1-8, 12-15

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

3

Date of the actual completion of the international search	Date of mailing of the international search report
14 August 1996	20.08.96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Espen, J

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 268, no. 26, 1993, BALTIMORE, MD US, pages 19476-19482, XP002010550 HIROYUKI SORIMACHI ET AL.: "A novel tissue-specific calpain species expressed predominantly in the stomach comprises two alternative splicing products with and without Ca2+-binding domain" see figure 2 ---	1-8, 12-15
X	BIOCHEMISTRY, vol. 27, 1988, EASTON, PA US, pages 8122-8128, XP002010551 SHINOBU IMAJOH ET AL.: "Molecular cloning of the cDNA for the large subunit of the High-Ca2+-Requiring form of human Ca2+-activated neutral protease" cited in the application see figure 2 ---	1-8, 12-15
Y	HUMAN MOLECULAR GENETICS, vol. 3, no. 2, February 1994, pages 285-293, XP002010552 FOUGEROUSSE F. ET AL.: "Mapping of a chromosome 15 region involved in limb girdle muscular dystrophy" cited in the application see the whole document ---	1-20
Y	JOURNAL OF MEDICAL GENETICS, vol. 30, 1993, pages 385-387, XP000578358 PASSOS-BUENO M. R. ET AL.: "Evidence of genetic heterogeneity in the autosomal recessive adult forms of limb-girdle muscular dystrophy following linkage analysis with 15q probes in Brazilian families" see the whole document ---	1-20
Y	GENOMICS, OCT 1994, 23 (3) P619-27, UNITED STATES, XP000578720 RICHARD I ET AL: "Regional localization of human chromosome 15 loci." see page 625 ---	1-20
A	INT. J. BIOCHEM., vol. 22, no. 8, 1990, pages 811-822, XP002010554 JOHNSON P. ET AL.: "Calpains (intracellular calcium-activated cysteine proteinases): structure-activity relationships and involvement in normal and abnormal cellular metabolism" see the whole document ---	

-/--

3

International Application No
PCT/EP 95/04575

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FASEB JOURNAL, vol. 8, no. 11, 1994, pages 814-822, XP002010555 TAKAOMI C. SAIDO ET AL.: "Calpain: new perspectives in molecular diversity and physiological-pathological involvement" see the whole document -----	

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
B2628A - FL		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP 95/ 04575	21/11/1995	22/11/1994
International Patent Classification (IPC) or national classification and IPC		
C12N15/57		
Applicant		
ASSOCIATION FRANCAISE CONTRE LES MYOPATHIES et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consists of a total of _____ sheets.

3. This report contains indications and corresponding pages relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19/06/1996	07.03.97
Name and mailing address of the IPEA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Netherlands Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	J. Espen
	Telephone No. +31 70 340-2625

Form PCT/IPEA/409 (cover sheet) (January 1994) (08/10/1996)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/EP95/04575

I. Basis of the report

1. This report has been drawn up on the basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*

☒ the international application as originally filed

☐ the description, pages , as originally filed
pages , filed with the demand
pages , filed with the letter of

☐ the claims, Nos. , as originally filed
Nos. , as amended under Article 19
Nos. , filed with the demand
Nos. , filed with the letter of

☐ the drawings, sheets / fig. , as originally filed
sheets / fig. , filed with the demand
sheets / fig. , filed with the letter of

2. The amendments have resulted in the cancellation of:

☐ the description, pages:

☐ the claims, Nos.

☐ the drawings, sheets / fig.

3. ☐ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2 (c)).

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty	Claims	9-20	YES
	Claims	1-8	NO
Inventive Step	Claims	9-11,15-20	YES
	Claims	1-8,12-14	NO
Industrial Applicability	Claims	1-20	YES
	Claims		NO

2. Citations and Explanations

1). The present international application relates to Limb-Girdle Muscular Dystrophy Type 2A (LGMD2A). LGMD2A is strongly correlated to mutations in the gene of the proteolytic enzyme calpain 3 (*CANP3*).
The genomic organization of the human *CANP3* gene was determined.
The above mentioned enzyme may also be referred to as 'p94' or 'nCL-1'.

2). The following document has been considered for the purposes of this report:

D1 THE JOURNAL OF BIOLOGICAL CHEMISTRY, Vol. 264, pp. 20106-20111,1989

3). The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of claims 1-8 is not new in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

Document D1 discloses the nucleotide sequence of the human *CANP3* gene and the deduced amino acid sequence (D1, Fig. 2).

In view of the above comment, D1 is novelty destroying for claims 1-8 (Art. 33 (2) PCT).
The involvement of the *CANP3* gene in the etiology of LGMD2 is an implicit feature of the above sequences, and does not render novel the claimed subject-matter.

4). The methods of claims 12-14 are novel (Art. 33 (2) PCT), however they do not involve an inventive step (Art. 33 (3) PCT) for the following reasons.

For the person skilled in the art, being aware of the known amino acid sequences of claims 5 to 7 or of the known nucleic acid sequences of claims 1-4, the carrying out of said methods required nothing out of the ordinary and thus involved no inventive skill, all being a matter of technical convenience. In addition, this was a matter of normal design procedures for which neither 'creative thinking' nor 'inventive talent' were necessary.

5). Claims 9-11,and 15-20 do meet the requirements of Art. 33 (2) and (3) for the following reasons:

Before the priority date of the present international application, it was neither disclosed nor suggested that the *CANP3* gene product, when mutated, is involved in the LGMD2 disease.

In consequence, the use of sequences related to the *CANP3* gene in the diagnostic of the LGMD2 disease or their use in the preparation of a pharmaceutical composition for the treatment of said disease could not be deduced in an obvious manner from the closest prior art document D1.

The industrial applicability of the present set of claims is acknowledged (Art. 33 (4) PCT).

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

-Claim 6 should read '..according to claim 5, ...'.
-Claim 18 should read '...by nucleic **acid** amplification...'

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
B2628A - FL		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 95/04575	21/11/95	22/11/94

Applicant

ASSOCIATION FRANCAISE CONTRE LES MYOPATHIES et al.

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [X] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing

   [ ] filed with the international application.

   [X] furnished by the applicant separately from the international application,

   [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.

   [ ] Transcribed by this Authority

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   LGMD GENE CODING FOR A CALCIUM DEPENDENT PROTEASE

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 95/04575

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/57 C12N9/64 A61K38/48 G01N33/50 C12Q1/68
C12N5/10 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N A61K G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

3

Date of the actual completion of the international search	Date of mailing of the international search report
14 August 1996	20.08.96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Espen, J

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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X	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 268, no. 26, 1993, BALTIMORE, MD US, pages 19476-19482, XP002010550 HIROYUKI SORIMACHI ET AL.: "A novel tissue-specific calpain species expressed predominantly in the stomach comprises two alternative splicing products with and without Ca2+-binding domain" see figure 2 ---	1-8, 12-15
X	BIOCHEMISTRY, vol. 27, 1988, EASTON, PA US, pages 8122-8128, XP002010551 SHINOBU IMAJOH ET AL.: "Molecular cloning of the cDNA for the large subunit of the High-Ca2+-Requiring form of human Ca2+-activated neutral protease" cited in the application see figure 2 ---	1-8, 12-15
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Y	GENOMICS, OCT 1994, 23 (3) P619-27, UNITED STATES, XP000578720 RICHARD I ET AL: "Regional localization of human chromosome 15 loci." see page 625 ---	1-20
A	INT. J. BIOCHEM., vol. 22, no. 8, 1990, pages 811-822, XP002010554 JOHNSON P. ET AL.: "Calpains (intracellular calcium-activated cysteine proteinases): structure-activity relationships and involvement in normal and abnormal cellular metabolism" see the whole document --- -/--	

3

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/57, 9/64, A61K 38/48, G01N 33/50, C12Q 1/68, C12N 5/10, A61K 48/00

A2

(11) International Publication Number: WO 96/16175

(43) International Publication Date: 30 May 1996 (30.05.96)

(21) International Application Number: PCT/EP95/04575

(22) International Filing Date: 21 November 1995 (21.11.95)

(30) Priority Data:
94402668.1 22 November 1994 (22.11.94) EP
(34) Countries for which the regional or international application was filed: GB et al.

(71) Applicant *(for all designated States except US)*: ASSOCIATION FRANÇAISE CONTRE LES MYOPATHIES [FR/FR]; 13, place de Rungis, F-75013 Paris (FR).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: BECKMANN, Jacques [FR/FR]; 95, rue de Paris, F-94220 Charenton-le-Pont (FR). RICHARD, Isabelle [FR/FR]; 72, rue de l'Essonne, F-91000 Evry (FR).

(74) Agents: GUTMANN, Ernest et al.; Ernest Gutmann - Yves Plasseraud S.A., 3, rue Chauveau-Lagarde, F-75008 Paris (FR).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: LGMD GENE CODING FOR A CALCIUM DEPENDENT PROTEASE

(57) Abstract

A nucleic acid sequence comprising: 1) the sequence represented in figure 8; or 2) the sequence represented in figure 2; or 3) a part of the sequence of figure 2 with the proviso that it is able to code for a protein having a calcium dependant protease activity involved in a LGMD2; or 4) a sequence derived from a sequence defined in 1), 2) or 3) by substitution, deletion or addition of one or more nucleotides with the proviso that said sequences still codes for said protease.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/57, 9/64, A61K 38/48, G01N 33/50, C12Q 1/68, C12N 5/10, A61K 48/00

A2

(11) International Publication Number: WO 96/16175

(43) International Publication Date: 30 May 1996 (30.05.96)

(21) International Application Number: PCT/EP95/04575

(22) International Filing Date: 21 November 1995 (21.11.95)

(30) Priority Data:
94402668.1 22 November 1994 (22.11.94) EP

(34) Countries for which the regional or international application was filed: GB et al.

(71) Applicant (for all designated States except US): ASSOCIATION FRANÇAISE CONTRE LES MYOPATHIES [FR/FR]; 13, place de Rungis, F-75013 Paris (FR).

(72) Inventors; and

(75) Inventors/Applicants (for US only): BECKMANN, Jacques [FR/FR]; 95, rue de Paris, F-94220 Charenton-le-Pont (FR). RICHARD, Isabelle [FR/FR]; 72, rue de l'Essonne, F-91000 Evry (FR).

(74) Agents: GUTMANN, Ernest et al.; Ernest Gutmann - Yves Plasseraud S.A., 3, rue Chauveau-Lagarde, F-75008 Paris (FR).

(81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: LGMD GENE CODING FOR A CALCIUM DEPENDENT PROTEASE

(57) Abstract

A nucleic acid sequence comprising: 1) the sequence represented in figure 8; or 2) the sequence represented in figure 2; or 3) a part of the sequence of figure 2 with the proviso that it is able to code for a protein having a calcium dependant protease activity involved in a LGMD2; or 4) a sequence derived from a sequence defined in 1), 2) or 3) by substitution, deletion or addition of one or more nucleotides with the proviso that said sequences still codes for said protease.

LGMD gene coding for a calcium dependent protease

The invention relates to the isolated gene coding for a calcium dependent protease belonging to the Calpain family which, when it is mutated, is a cause of a disease called Limb-Girdle Muscular Dystrophy (LGMD).

The term limb-girdle muscular dystrophy (LGMD) was first proposed by Walton and Nattrass (1954) as part of a classification of muscular dystrophies. LGMD is characterised by progressive symmetrical atrophy and weakness of the proximal limb muscles and by elevated serum creatine kinase. Muscle biopsies demonstrate dystrophic lesions and electromyograms show myopathic features. The symptoms usually begin during the first two decades of life and the disease gradually worsens, often resulting in loss of walking ability 10 or 20 years after onset (Bushby, 1994). Yet, the precise nosological definition of LGMD still remains unclear. Consequently, various neuromuscular diseases such as facioscapulohumeral, Becker muscular dystrophies and especially spinal muscular atrophies have been occasionally classified under this diagnosis. For example, a recent study (Arikawa et al., 1991) reported that 17% (out of 41) of LGMD patients showed a dystrophinopathy. These issues highlight the difficulty in undertaking an analysis of the molecular and genetic defect(s) involved in this pathology.

Attempts to identify the genetic basis of this disease go back over 35 years. Morton and Chung (1959) estimated that "the frequency of heterozygous carrier ... is 16 per thousand persons". The same authors also stated that "the segregation analysis gives no evidence on whether these genes in different families are allelic or at different loci". Both autosomal dominant and recessive transmission have been reported, the latter being more common with an estimated prevalence of 10^{-5} (Emery, 1991). The localisation of a gene for a recessive form on chromosome 15 (LGMD2A, MIM 253600; Beckmann et al., 1991) provided the definitive proof that LGMD is a specific genetic entity. Subsequent genetic analyses confirmed this chromosome 15 localisation (Young et al., 1992; Passos-Bueno et al., 1993), the latter group demonstrating genetic heterogeneity of this disease. Although a recent study localised a second mutant

gene to chromosome 2 (LGMD2B, MIM 253601; Bashir et al., 1994). there is evidence that at least one other locus can be involved.

Genetic analyses of the LGMD2 kindreds revealed unexpected findings. First genetic heterogeneity was demonstrated in the highly inbred Indiana Amish community. Second although the Isle of la Réunion families were thought to represent a genetic isolate, at least 6 different disease haplotypes were observed , providing evidence against the hypothesis of a single founder effect (Beckmann et al., 1991) in this inbred population.

The nonspecific nosological definition, the relatively low prevalence and genetic heterogeneity of this disorder limit the number of families which can be used to restrict the genetic boundaries of the LGMD2A interval. Cytogenetic abnormalities, which could have helped to focus on a particular region, have not been reported. Immunogenetic studies of dystrophin-associated proteins (Matsumura et al., 1993) and cytoskeletal or extracellular matrix proteins such as a merosin (Tomé et al., 1994) failed to demonstrate any deficiency. In addition, there is no known specific physiological feature or animal model that could help to identify a candidate gene. Thus, there is no alternative to a positional cloning strategy.

It is established that the LGMD2 chromosomal region is localized on chromosome 15 as 15q15.1 - 15q21.1 region (Fougerousse et al., 1994).

Construction and analysis of a 10-12 Mb YAC contig (Fougerousse et al., 1994) permitted the mapping of 33 polymorphic markers within this interval and to further narrow the LGMD2A region to between D15S514 and D15S222. Furthermore, extensive analysis of linkage disequilibrium suggested a likely position for the gene in the proximal part of the contig.

The invention results from the construction of a partial cosmid map and the screening by cDNA selection (Lovett et al., 1991 ; Tagle et al., 1993) for muscle-expressed sequences encoded by this interval led to the identification of a number of potential candidate genes. One of these, previously cloned by Sorimachi et al. (1989), encodes a muscle specific protein, nCL1 (novel Calpain Large subunit 1), which belongs to the calpain family (CANP, calcium-activated neutral protease; EC 3.4.22.17), and appeared to be a functional candidate gene for this disease.

Calpains are non-lysosomal intracellular cysteine proteases which require calcium for their catalytic activities (for a review see Croall D.E. et al, 1991). The mammalian calpains include two ubiquitous proteins CANP1 and CANP2 as well as tissue-specific proteins. In addition to the muscle specific nCL1, stomach specific nCL2 and nCL2' proteins have also been described; these are derived from the same gene by alternative splicing. The ubiquitous enzymes consist of heterodimers with distinct large subunits associated with an common small subunit ; the association of tissue-specific large subunits with a small subunit has not yet been demonstrated. The large subunits of calpains can be subdivided into 4 protein domains. Domains I and III, whose functions remain unknown, show no homology with known proteins. Domain I, however, seems important for the regulation of the proteolytic activity. Domain II shows similarity with other cysteine proteases, sharing histidine, cysteine and asparagine residues at its active sites. Domain IV comprises four EF-hand structures which are potential calcium binding sites. In addition, three unique regions with no known homology are present in the muscle-specific nCL1 protein, namely NS, IS1 and IS2, the latter containing a nuclear translocation signal. These regions may be important for the muscle specific function of nCL1.

It is usually accepted that muscular dystrophies are associated with excess or deregulated calpaïns, and all the known approaches for curing these diseases are the use of antagonists of these proteases ; examples are disclosed in EP 359309 or EP 525420.

The invention results from the finding that, on the opposite to all these hypothesis, the LGMD2 disease is strongly correlated to the defect of a calpaïn which is expressed in healthy people.

The invention relates to the nucleic acid sequence such as represented in Figure 2 coding for a Ca^{++} dependent protease, or calpaïn, which is involved in LGMD2 disease, and more precisely LGMD2A. It also relates to a part of this sequence provided it is able to code for a protein having a calcium-dependent protease activity involved in LGMD2, or a sequence derived from one of the above sequences by substitution, deletion or addition of one or more nucleotides provided that said sequence is still coding for said protein, all the nucleic acids yielding a sequence complementary to a sequence as defined above.

The genomic organisation of the human nCL1 gene has been determined by the inventors, and consists of 24 exons and extends over 40 kb as represented in Figure 8, and is also a part of the invention. About 35 kb of this gene have been sequenced. A systematic screening of this gene in LGMD2A families led to the identification of 14 different mutations, establishing that a number of independent mutational events in nCL1 are responsible for LGMD2A. Furthermore, this is the first demonstration of a muscular dystrophy resulting from an enzymatic rather than a structural defect.

In the present specification, CANP3 means the protein which is a Ca^{++} dependent protease, or calpaïn, and coded by the nCL1 gene on chromosome 15.

The invention relates also to a protein, called CANP3, consisting in the amino acid sequence such as represented in figure 2 and which is involved, when mutated, in the LGMD2 disease.

The cDNA of the gene coding for CANP3, which is coding for the protein, is also represented in Figure 2, and is a part of the invention.

The protein coded by this DNA is CANP3, a calcium-dependent protease belonging to the Calpaïn family.

Are also included in the present invention the nucleic acid sequences derived from the cDNA of Figure 2 by one or more substitutions, deletions, insertions, or by mutations in 5' or 3' non coding regions or in splice sites, provided that the translated protein has the protease, calcium-dependent activity, and when mutated, induce LGMD2 disease.

The nucleic acid sequence encoding the protein might be DNA or RNA and be complementary to the nucleic and sequence represented in Figure 2.

The invention also relates to a recombinant vector including a DNA sequence of the invention, under the control of a promoter allowing the expression of the calpaïn in an appropriate host cell.

A procaryotic or eucaryotic host cell transformed by or transfected with a DNA sequence comprising all or part of the sequence of Figure 2 is a part of the invention.

Such a host cell might be either :

- a cell which is able to secrete the protein and. this recombinant protein might be used as a drug to treat the LGMD2, or

- a packaging cell line transfected by a viral or retroviral vector ; the cell lines bearing recombinant vector might be used as a drug for gene therapy of LGMD2.

All the systems used today for gene therapy including adenoviruses and retroviruses and others described for example in « l'ADN médicament », (John Libbey, Eurotext, 1993), and bearing one of the DNA sequence of the invention are included herein by reference.

The examples hereunder and attached figures indicate how the structure of the gene was established, and how relationship between the gene and the LGMD was established.

Legend of the figures :

Figure 1:

A) Genomic organisation of the nCL1 gene

The gene covers a 40 kb region of which 35 were sequenced (Accession number pending). Introns and exons are drawn to scale, the latter being indicated by numbered vertical bars. The first intron is the largest one and remains to be fully sequenced. Position of intragenic microsatellites are indicated by asterisks. Arrows indicate the orientation of Alu (closed) and of Mer2 (greyed) repeat sequences.

B) *EcoR*I restriction map

An *EcoR*I (E) restriction map of this region was established with the help of cosmids from this region. The location of nCL1 gene is indicated as a black bar. The size of the corresponding fragments are indicated and are underlined when determined by sequence analysis.

C) Cosmid map of the nCL1 gene region.

Cosmids were from a cosmid library constructed by subcloning YAC 774G4 (Richard in preparation) and are presented as lines. Dots on lines indicate positive STSs (indicated in boxed rectangles). A minimum of three cosmids cover the entire gene. T3,T7

Figure 2: Sequence of the human nCL1 cDNA (B) , and the flanking 5' (A) and 3' (C) genomic regions.

A) and C) The polyadenylation signal and putative CAAT, TATAA sites are boxed. Putative Sp1 (position -477 to -472), MEF2 binding sites (-364 to -343) and CArG box (-685 to -672) are in bold. The Alu sequence present in the 5' region is underlined.

B) The corresponding amino acids are shown below the sequence. The coding sequence between the ATG initiation codon and the TGA stop codon is 2466 bp, encoding for a 821 amino acid protein. The adenine in the first methionine codon has been assigned position 1. Locations of introns within the nCL1 gene are indicated by arrowheads. Nucleotides which differ from the previously published ones are indicated by asterisks.

Figure.3: Alignments of amino acid sequences of the muscle-specific calpains.

The human nCL1 protein is shown on the first line. The 3 muscle-specific sequences (NS, IS1 and IS2) are underlined. The second line corresponds to the rat sequence (Accession no P). The third and fourth lines show the deduced amino acid sequences encoded by pig and bovine Expressed Sequences Tagged (GenBank accession no U05678 and no U07858, respectively). The amino acids residues which are conserved among all known members of the calpains are in reverse letters. A period indicates that the same amino acid is present in the sequence. Letters refer to the variant amino acid found in the homologous sequence. Position of missense mutations are given as numbers above the mutated amino acid.

Figure 4: Distribution of the mutations along nCL1 protein structure.

A) Positions of the 23 introns are indicated by vertical bars in relation to the corresponding amino acid coordinates.

B) The nCL1 protein is depicted showing the four domains (I, II, III, IV) and the muscle specific sequences (NS, IS1 and IS2). The position of missense mutations within nCL1 domain are indicated by black dots. The effect of nonsense and frameshift mutations are illustrated as truncated lines, representing the extent of protein synthesised. Name of the corresponding families are indicated on the left of the line. The out of frame ORF is given by hatched lines.

Figure 5: Northern blot hybridisation of a nCL1 clone

A mRNA blot (Clontech) containing 2 µg of poly(A)+ RNA from each of eight human tissues was hybridised with a nCL1 genomic clone spanning exons 20 and 21. The latter detects a 3.6 kb mRNA present only in a line corresponding to the skeletal muscle mRNA.

Figure 6: Representative mutations identified by heteroduplex analysis.

Examples of mutation screening by heteroduplex analysis. Pedigree B505 shows the segregation of two different mutations in exon 22.

Figure 7: Homozygous mutations in the nCL1 gene

Detection by sequencing of mutations in exons 2 (a), 8 (b), 13 (c) and 22 (d). Sequences from a healthy control are shown above each mutant sequence. Asterisks indicate the position of the mutated nucleotides. The consequences on codon and amino acid residues are indicated on the left of the figure together with the name of the family.

Figure 8 : Structure of nCL1 gene

Figure 8A represents the 5' part of the gene with exon 1.

Figure 8B represents the part of the gene including exons 2 to 8,

Figure 8C represents the part of the gene including exon 9,

Figure 8D represents the part of the gene including exons 10 to 24 including the 3' non transcribed region.

EXAMPLES

EXAMPLE 1

Localisation of the nCL1 within the LGMD2A interval

Detailed genetic and physical maps of the LGMD2A region were constructed (Fougerousse et al., 1994), following the primary linkage assignment to 15q (Beckmann et al., 1991). The disease locus was bracketed between the D15S129 and D15S143 markers, defining the cytogenetic boundaries of the LGMD2A region as 15q15.1-15q21.1 (Fougerousse et al., 1994). Construction and analysis of a 10-12 Mb YAC contig (Fougerousse et al., 1994) permitted us to map 33 polymorphic markers within this interval and to further narrow the LGMD2A region to between D15S514 and D15S222.

The nCL1 gene had been localised to chromosome 15 by hybridisation with sorted chromosomes and by Southern hybridisation to DNA from human-mouse cell hybrids (Ohno et al., 1989).cDNA capture using YACs from the LGMD2A interval allowed the identification of thirteen positional candidate genes. nCL1 was one of the two transcripts identified that showed muscle-specific expression as evidenced by northen blot analysis.The localisation was further confirmed by STS (for Sequence Tagged Site) assays. Primers used for the localisation of the nCL1 gene are P94in2, P94in13 and pcr6a3, as shown in Figure 1 and their characteristics being defined in Table 1.

Table 1: PCR primers used for localisation of the nCL1 gene.

Primer name	Primer sequence (5'-3')	Position within the cDNA	Annealing temp (°C)	PCR product size on	
				cDNA	genomic DNA
P94in2	ATGGAGCCAACAGAACTGA C GTATGACTCGGAAAAGAAG GT	341-360 428-448	58	108	1758
P94in13	TAAGCAAAAGCAGTCCCCA C TTGCTGTTCCTCACTTTCCT G	1893-1912 1936-1956	58	64	1043
P94-6a3	GTTTCATCTGCTGCTTCGTT CTGGTTCAGGCATACATGG T	2342-2361 2452-2471	56	130	818
P94ex1ter	TTCTTTATGTGGACCCTGAG TT ACGAACTGGATGGGGAACT	218-239 275-293	55	76	76

These primers are designed from different parts of the published human cDNA sequence (Sorimachi et al., 1989), and were used for an STS content screening on DNA from three chromosome 15 somatic cell hybrids and YACs from the LGMD2A contig. The results positioned the gene in a region previously defined as 15q15.1-q21.1 and on 3 YACs (774G4, 926G10, 923G7) localised in this region. The relative positions of STSs along the LGMD2A contig allowed to localise the gene between D15S512 and D15S488, in a candidate region suggested by linkage disequilibrium studies.

The same primers as above were used to screen a cosmid library from YAC 774G4. A group of 5 cosmids was identified (Fig. 1). Experiments with another nCL1 primer pair (P94ex1ter; Table 1) established that these cosmids cover all nCL1 exons except number 1, and that a second group of 4 cosmids contain this

exon (Fig. 1). A minimal set of three overlapping cosmids (2G8-2B11-1F11) covers the entire gene (Figure 1). DNA from these cosmids was used to construct an *EcoR*I restriction map of this region (Figure 1B).

EXAMPLE 2

Determination of the nCL1 gene sequence

Most of the sequences were obtained through shotgun sequencing of partial digests of cosmid 1F11 subcloned in M13 and bluescript vectors, and by walking with internal primers. The sequence assembly was made using the XBAP software of the Staden package (Staden) and was in agreement with the restriction map of the cosmids. Sequences of exon 1 and adjacent regions were obtained by sequencing cosmid DNA or PCR products from human genomic DNA. The first intron is still not fully sequenced, but there is evidence that it may be between 10 to 16 kb in length (based on hybridisation of restriction fragments; data not shown). The entire gene, including its 5' and 3' regions, is more than 40 kb long, and shown in Figure 8.

a) the cDNA sequence

The used technology allows the implementation of the published human cDNA sequence of nCL1 (Sorimachi 1989). It contains the missing 129 bases corresponding to the N-terminal 43 amino acids (Figure 2). It also differs from it at 12 positions. Three of which occur at third base positions of codons and preserve the encoded amino acid sequence. The other 9 differences lead to changes in amino-acid composition (Figure 2). As these different exons were sequenced repeatedly on at least 10 distinct genomes, we are confident that the sequence of Fig. 2 represents an authentic sequence and does not contain minor polymorphic variants. Furthermore, these modifications increase the local similarity with the rat nCL1 amino acid sequence (Sorimachi), although the overall similarity is still 94 %.

The ATG numbered 1 in Figure 2 is the translation initiation site based on homology with the rat nCL1, and is within a sequence with only 5 nucleotides out of 8 in common with the Kosak consensus sequence (Kosak M, 1984). Putative CCAAT and TATA boxes were observed 590, 324, (CCAAT) and 544 or 33 bp (TATA) upstream of the initiating ATG codon, respectively (Bucher, 1990). A GC-box binding the Sp1 protein (Dynan et al., 1983) was identified at position -477.

Consensus sequences corresponding to potential muscle-specific regulatory elements were identified (Fig. 2). These include a myocyte-specific enhancer-binding factor 2 (MEF2) binding site (Cserjesi P. 1991), a CArG box (Minty A. 1986) and 6 E-boxes (binding sites for basic Helix-Loop-Helix proteins frequently found in members of MyoD family; Blackwell et Weintraub, 1990). The functional significance of these putative transcription factor binding sites in the regulation of nCL1 gene expression remains to be established.

Two potential AAUAAA polyadenylation signals, were identified 520 and 777 bp downstream of the TGA stop codon. The sequencing of a partial nCL1 cDNA containing a polyA tail, demonstrated that the first AAUAAA is the polyadenylation signal. The latter is embedded in a region well conserved with the rat nCL1 sequence and is followed after 4 bp by a G/T cluster, present in most genes 3' of the polyadenylation site (Birnstiel et al., 1985). The 3'-untranslated region of the nCL1 mRNA is 565 bp long. The predicted length of the cDNA should therefore be approximately 3550 or 3000 bp.

b) Comparison with calpain

The sequence of the human nCL1 gene was compared to those of other calpains thereof (Figure 3). The most telling comparisons are with the homologous rat (Accession no J05121), bovine (Accession no U07858) and porcine (Accession no U05678) sequences. The accession numbers refers to those or international genebanks, such as GeneBank (N.I.H.) or EMBL Database (EMBL, Heidelberg). High local similarities between the human and rat DNA sequences are even observed in the 5' (75%) or in different parts of the 3' untranslated regions (over 60%) (data not shown). The high extent of sequence homology manifested by the human and rat nCL1 gene in their untranslated regions is suggestive of evolutionary pressures on common putative regulatory sequences.

c) Genomic organisation of the nCL1 gene

A comparison of the published nCL1 human cDNA (Sorimachi et al., 1989) with the corresponding genomic sequence led to the identification of 24 exons ranging in length from 12 bp (exon 13) to 309 bp (exon 1), with a mean size of 100 bp (Figure 1). The size of introns ranges from 86 bp to about 10-16 kb for intron 1.

The intron-exon boundaries as shown in Table 2 exhibit close adherence to 5' and 3' splice site consensus sequences (Shapiro and Senapathy, 1987).

Table 2: Sequences at the intron-exon junctions. A score expressing adherence to the consensus was calculated for each site according to Shapiro and Senapathy (1987). Sequences of exons and introns are in upper and lower cases, respectively. Size of exons are given in parenthesis.

splice donor site	score (%)	Intron	score (%)	splice acceptor site	Exon
					Exon 1 (309 bp) ->
...CTCCGgtgagt...	88.5	<-Intron 1->	99.0	...ttttgttcacagGAAAT...	Exon 2 (70 bp) ->
...GCTAGgtagga...	83.5	<-Intron 2->	90.0	...gtgtctgcctgcagGGGAC...	Exon 3 (119 bp) ->
...TCCAGgtgagg...	92	<-Intron 3->	81.5	...acgcttctgtgcagTTCTG...	Exon 4 (134 bp) ->
...GCTAAgtaagc...	82	<-Intron 4->	81.5	...atcctctctctaagGCTCC...	Exon 5 (169 bp) ->
...TTGATgtaagt...	87	<-Intron 5->	79.5	...ccatcgggcctcagGATGG...	Exon 6 (144 bp) ->
...CCCGGgtgtgt...	77.5	<-Intron 6->	91	...ttactgctctacagACAAT...	Exon 7 (84 bp) ->
..ATGAGgtaagc...	94	<-Intron 7->	78.5	...tctgtgtgcttaagGTCCC...	Exon 8 (86 bp) ->
..GATAGgtaggt...	89	<-Intron 8->	91.5	...cattttcccaccagATGGA...	Exon 9 (78 bp) ->
...TTCTGgtgagt...	88	<-Intron 9->	92	...ttccaacctctcagGATGT...	Exon 10 (161 bp) ->
...CCCAGgtggga...	80	<-Intron 10->	68.5	...ttctgggggtgcagATACT...	Exon 11 (170 bp) ->
...ACGAGgtgtgt...	85.5	<-Intron 11->	86	...tgtttcttctcaagGTTCC...	Exon 12 (12 bp) ->
..AAGAGgtatag...	70	<-Intron 12->	87	...tccccatctctcagATGCA...	Exon 13 (209 bp) ->
...TCTGAgtgagt...	76.5	<-Intron 13->	97	...tgtattcctcacagGGAAG...	Exon 14 (37 bp) ->
...CAGTGgtgagt...	89	<-Intron 14->	93.5	...cttttcttatgcagAAAAA...	Exon 15 (18 bp) ->
...CCAAGgtaggt...	89	<-Intron 15->	87	...cctcctctctccagCCCAT...	Exon16 (114 bp) ->
...CACAGgtgtct...	80	<-Intron 16->	88	...ttgtgcctccacagCCACA...	Exon 17 (78 bp) ->
...GAGATgtgagt...	84	<-Intron 17->	92.5	...ccctcctcctcagGACAT...	Exon 18 (58 bp) ->
..CAAACgtgagt...	83	<-Intron 18->	90	...ctccatcccccccagACAAG...	Exon 19 (65 bp) ->
..TGGATgtatcc..	56	<-Intron 19->	88	...cctcccctcctccagACAGA...	Exon 20 (69 bp) ->
...GGCAGgtggga...	80	<-Intron 20->	94	...ttttctattgccagAAATA...	Exon 21 (79 bp) ->
.CGCAGgtgctg..	66	<-Intron 21->	91	...ggtcccctccacagGATTC...	Exon 22 (117 bp) ->

...GTTCAgtaagt...	79	<-Intron 22->	93.5	...gcattctttcacagGAGCT...	Exon 23 (59 bp) ->
...TGGAGgtaaag...	81	<-Intron 23->	79	...gggactctttcagTGGCT...	Exon 24 (27 bp) ->

When the genomic sequence was submitted to GRAIL analysis (Uberbacher et al., 1991), 11 exons were correctly recognised, 4 were not identified, 6 were inadequately defined and 2 were too small to be recognised (data not shown).

As already noted, the nCL1 gene has three unique sequence blocks, NS (amino acid residues 1 to 61), IS1 (residues 267 to 329) and IS2 (residues 578 to 653). It is interesting to note that each of these sequences, as well as the nuclear translocation signal inside IS2, are essentially flanked by introns (Fig. 4). The exon-intron organisation of the human nCL1 is similar to that reported for the chicken CANP (the only other large subunit calpain gene whose genomic structure is known; (Emori et al., 1986).

Four microsatellite sequences were identified. Two of them are in the distal part of the first intron: an $(AT)_{14}$ and an previously identified mixed-pattern microsatellite, S774G4B8, which was demonstrated to be non polymorphic (Fougerousse et al., 1994). A $(TA)_7(CA)_4(GA)_{13}$ was identified in the second intron and genotyping of 64 CEPH unrelated individuals revealed two alleles (with frequencies of 0.10 and 0.90). The fourth microsatellite is a mixed $(CA)_n(TA)_m$ repeat present in the 9th intron. The latter and the $(AT)_{14}$ repeat have not been investigated for polymorphism. Fourteen repetitive sequences of the Alu family and one Mer2 repeat were identified in the nCL1 gene (Fig. 1C), which has, thus, on the average one Alu element per 2.5 kb.

Southern blot experiments (Ohno et al., 1989) and STS screening (data not shown) suggest that there is but one copy per genome of this member of the calpaïn family.

EXAMPLE 3

Expression of the nCL1 gene

The pattern of tissue-specificity was investigated by northern blot hybridisation with a genomic subclone probe from cosmid 1F11 spanning exons 20 and 21.There is no evidence for the existence of an alternatively spliced form of nCL1, although this cannot be excluded. A transcript of about 3.4-3.6 kb was

detected in skeletal muscle mRNA (Figure 5). This size therefore favours that the position -544 is the functional TATA box.

Transcription studies suggested that it is an active gene rather than a pseudogene and its muscle-specific pattern of expression is consistent with the phenotype of this disorder (Sorimachi et al., 1989 and Figure 5).

EXAMPLE 4

Mutation screening

nCL1 fulfils both positional and functional criteria to be a candidate gene for LGMD2A. To evaluate its role in the etiology of this disorder, nCL1 was systematically screened in 38 LGMD2 families for the presence of nucleotide changes using a combination of heteroduplex (Keen et al., 1991) and direct sequence analyses.

PCR primers were designed to specifically amplify the exons and splice junctions and also the regions containing the putative CAT, TATA boxes and the polyadenylation signal of the gene as shown in Table 3.

Table 3: PCR primers used for the analysis of the nCL1 gene in LGMD patients.

amplified region	Primer sequences (5'-3')	Size (bp)	Annealing temp. (°C)
promotor	TTCAGTACCTCCCGTTCACC GATGCTTGAGCCAGGAAAAC	296	59
exon 1	CTTTCCTTGAAGGTAGCTGTAT GAGGTGCTGAGTGAGAGGAC	438	60
exon 2	ACTCCGTCTCAAAAAAATACCT ATTGTCCCTTTACCTCCTGG	239	57
exon 3	TGGAAGTAGGAGAGTGGGCA GGGTAGATGGGTGGGAAGTT	354	58
exon 4	GAGGAATGTGGAGGAAGGAC TTCCTGTGAGTGAGGTCTCG	292	59
exon 5	GGAACTCTGTGACCCCAAAT TCCTCAAACAAAACATTCGC	325	56
exon 6	GTTCCCTACATTCTCCATCG GTTATTTCAACCCAGACCCTT	315	57
exon 7	AATGGGTTCTCTGGTTACTGC AGCACGAAAAGCAAAGATAAA	333	56
exon 8	GTAAGAGATTTGCCCCCCAG TCTGCGGATCATTGGTTTTG	321	58
exon 9	CCTTCCCTTCTTCCTGCTTC CTCTCTTCCCCACCCTTACC	173	56
exon 10	CCTCCTCACCTGCTCCCATA TTTTTCGGCTTAGACCCTCC	251	56
exon 11	TGTGGGGAATAGAAATAAATGG CCAGGAGCTCTGTGGGTCA	355	57
exon 12	GGCTCCTCATCCTCATTCACA GTGGAGGAGGGTGAGTGTGC	312	61
exon 13	TGTGGCAGGACAGGACGTTC	337	60

exon 14	TTCAACCTCTGGAGTGGGCC CACCAGAGCAAACCGTCCAC	230	61
exon 15	ACAGCCCAGACTCCCATTCC TTCTCTTCTCCCTTCACCCT	225	57
exon 16	ACACACTTCATGCTCTCTACCC CCGCCTATTCCTTTCCTCTT	331	56
exon 17	GACAAACTCCTGGGAAGCCT ACCTCTGACCCCTGTGAACC	270	61
exon 18	TGTGGATTTGTGTGCTACGC CATAAATAGCACCGACAGGGA	258	59
exon 19	GGGATGGAGAAGAGTGAGGA TCCTCACTCTTCTCCATCCC	159	57
exons 20-21	ACCCTGTATGTTGCCTTGG GGGGATTTTGCTGTGTGCTG	333	61
exon 22	ATTCCTGCTCCCACCGTCTC CACAGAGTGTCCGAGAGGCA	282	57
exons 22-23	GGAGATTATCAGGTGAGATGCC CAGAGTGTCCGAGAGGCAGGG	608	61
exon 24	CGTTGACCCCTCCACCTTGA GGGAAAACATGCACCTTCTT	375	58
polyadenylation signal	TAGGGGGTAAAATGGAGGAG ACTAACTCAGTGGAATAGGG GGAGCTAGGATAGCTCAAT	413	56

PCR products made on DNA from blood of specific LGMD2A patients were then subjected either to heteroduplex analysis or to direct sequencing, depending on whether the mutation, based on haplotype analysis, was expected to be homozygous or heterozygous, respectively. It was occasionally necessary to clone the PCR products to precisely identify the mutations (i.e., for microdeletions or insertions and for some heterozygotes). Disease-associated mutations are summarised in Table 4 hereunder and their position along the protein is shown in Fig. 4.

Table 4: nCL1 mutations in LGMD2A families.

Codons and amino acid positions are numbered on the basis of the cDNA sequence starting from ATG.

Exon	Families	Nucleotide position	Nucleotide change	Amino acid position	Amino acid change	Restriction si
2	B519*	328	CGA->TGA	110	Arg->stop	
4	M42	545	CTG -> CAG	182	Leu->Gln	
4	M1394: M2888	550	CAA -> CA	184	frameshift	
5	M35: M37	701	GGG -> GAG	234	Gly->Glu	

6	M32	945	CGG -> CG	315	frameshift	-SmaI
8	M2407*	1061	GTG -> GGG	354	Val-> Gly	
8	M1394	1079	TGG -> TAG	360	Trp->stop	-BstnI, -Eco
11	M2888	1468	CGG -> TGG	490	Arg->Trp	
13	R12*	1715	CGG -> CAG	572	Arg->Gln	-MspI
19	R27	2069-2070	deletion AC	690	frameshift	
21	R14; R17	2230	AGC -> GGC	744	Ser->Gly	-AluI
22	A*; B501*; M32	2306	CGG -> CAG	769	Arg->Gln	
22	B505	2313-2316	deletion AGAC	771-772	frameshift	
22	R14; B505	2362-2363	AG -> TCATCT	788	frameshift	

The first letter of the family code refers to the origin of the population B= Brazil, M= metropolitan France, R = Isle of La Réunion, A= Amish.

Each mutation was confirmed by heteroduplex analysis, by sequencing of both strands in several members of the family or by enzymatic digestion when the mutation resulted in the modification of a restriction site. Segregation analyses of the mutations, performed on DNAs from all available members of the families, confirmed that these sequence variations are on the parental chromosome carrying the LGMD2A mutation. To exclude the possibility that the missense substitutions might be polymorphisms, their presence was systematically tested in a control population: none of these mutations was seen among 120 control chromosomes from the CEPH reference families.

EXAMPLE 5 :

Analysis of families genes, chromosome-15 ascertained families

The initial screening for causative mutations was performed on families, each containing a LGMD gene located on chromosome 15. These included families from the Island of La Réunion (Beckmann et al., 1991), from the Old Order Amish from northern Indiana (Young et al., 1992,) and 2 Brazilian families (Passos Bueno et al., 1993).

a) Reunion Island families

Genealogical studies and geographic isolation of the families from the Isle of La Réunion were suggestive of a single founder effect. Genetic analyses are,

however, inconsistent with this hypothesis as the families present haplotype heterogeneity. At least, six different carrier chromosomes are encountered, (with affected individuals in several families being compound heterozygotes). Distinct mutations corresponding to four of these six haplotypes have been identified thus far.

In family R14, exons 13, 21 and 22 showed evidence for sequence variation upon heteroduplex analysis (Fig. 6). Sequencing of the associated PCR products revealed (i) a polymorphism in exon 13, (ii) a missense mutation (A->G) in exon 21 transforming the Ser^{744} residue to a glycine in the loop of the second EF-hand in domain IV of the protein (Figure 4), and (iii) a frameshift mutation in exon 22. The exon 21 mutation and the polymorphism in exon 13 form an haplotype which is also encountered in family R17. Subcloning of the PCR products was necessary to identify the exon 22 mutation. Sequencing of several clones revealed a replacement of AG by TCATCT (data not shown). This frameshift mutation causes premature termination at nucleotide 2400 where an in frame stop codon occurs (Figure. 4).

The affected individuals in family R12 are homozygous for all markers of the LGMD2A interval (Allamand, submitted). Sequencing of the PCR products of exon 13 revealed a G to A transition at base 1715 of the cDNA resulting in a substitution of glutamine for Arg^{572} (Figure. 7) within domain III, a residue which is highly conserved throughout all known calpains. This mutation, detectable by loss of *Msp*I restriction site, is present only in this family and in no other examined LGMD2A families or unrelated controls.

In family R27, heteroduplex analysis followed by sequencing of the PCR products of an affected child revealed a two base pair deletion in exon 19 (Figure. 6 and table 4). One AC out of three is missing at this position of the sequence, producing a stop codon at position 2069 of the cDNA sequence (Figure 4).

b) Amish families

As expected, due to multiple consanguineous links, the examined LGMD2A Northern Indiana Amish patients were homozygous for the haplotype on the chromosome bearing the mutant allele (Allamand, submitted). A (G->A) missense mutation was identified at nucleotide 2306 within exon 22 (Fig. 7). The

resulting codon change is CGG to CAG, transforming Arg^{769} to glutamine. This residue, which is conserved throughout all members of the calpain family in all species, is located in domain IV of the protein within the 3rd EF-hand at the helix-loop junction (ref). This mutation was encountered in a homozygous state in all patients from 12 chromosome 15-linked Amish families, in agreement with the haplotype analysis. We also screened six Southern Indiana Amish LGMD families, for which the chromosome 15 locus was excluded by linkage analyses (Allamand ESHG, submitted, ASHG 94). As expected, this nucleotide change was not present in any of the patients from these families, thus confirming the genetic heterogeneity of this disease in this genetically related isolate.

c) Brazilian families

As a result of consanguineous marriages, two Brazilian families (B501, B519) are homozygous for extended LGMD2A carrier haplotypes (data not shown). Sequencing PCR products from affected individuals of these families demonstrated that family B501 has the same exon 22 mutation found in northern Indiana Amish patients (Figure 7), but embedded in a completely different haplotype. In family B519, the patients carry a C to T transition in exon 2, replacing Arg^{328} with a TGA stop codon (Figure 7), thus leading, presumably, to a very truncated protein (Figure 4).

d) Analysis of other LGMD families

Having validated the role of the candidate gene in the chromosome 15 ascertained families, we next examined by heteroduplex analysis LGMD families for which linkage data were not informative. These included one Brazilian (B505) and 13 metropolitan French pedigrees.

Heteroduplex bands were revealed for exons 1, 3, 4, 5, 6, 8, 11, 22 of one or more patients (Figure 6). Of all sequence variants, 10 were identified as possible pathogenic mutations (5 missense, 1 nonsense and 4 frameshift mutations) and 3 as polymorphisms with no change of amino acid of the protein. All causative mutations identified are listed in Table 4 here-above. Identical mutations were uncovered in apparently unrelated families. The mutations shared by families M35 and M37, and M2888 and M1394, respectively, are likely to be the consequence of independent events since they are embedded in different marker haplotypes. In contrast, it is likely that the point mutation in exon

22 of the Amish and in the M32 kindreds corresponds to the same mutational event as both chromosomes share a common four marker haplotype (774G4A1-774G4A10-774G454D-774G4A2) around nCL1 (data not shown), possibly reflecting a common ancestor. The same holds true for the AG to TCATCT substitution mutation encountered in exon 22 in families B505 and R14. The exon 8 (T->G) transversion is present in the two carrier chromosomes of M2407, the only metropolitan family homozygous by haplotype, possibly reflecting an undocumented consanguinity. For some families, no disease-causing mutation has been detected thus far (M40 for example).

In addition to the polymorphism present in exon 13 in families R14 and R17 (position 668) and in the intragenic microsatellites, four additional neutral variations were detected: a (T->C) transition at position 96, abolishing a *Dde*I restriction site in exon 1 in M31; a (C->T) transition in exon 3 (position 495) in M40 and in M37 forming a haplotype with the exon 5 mutation (in the former family, this polymorphism does not cosegregate with the disease); a (T->C) transition in the paternally derived promotor in M42 at position -428, which was also evidenced in healthy controls; and a variable poly(G) in intron 22 close to the splice site in families R20, R11, R19, M35 and M37. The latter is also present in the members of the CEPH families, but is not useful as a genetic marker as the visualisation and interpretation of mononucleotide repeat alleles is difficult.

In total, sixteen independent mutational events representing fourteen different mutations were identified. All mutations cosegregate with the disease in LGMD2A families. The characterised morbid calpain alleles contain nucleotide changes which were not found in alleles from normal individual. The discovery of two nonsense and five frameshift mutations in nCL1 supports the hypothesis that a deficiency of this product causes LGMD2A. All seven mutations result in a premature in-frame stop codon, leading to the production of truncated and presumably inactive proteins (Figure 4). Evidences for the morbidity of the missense mutations come from (1) the relative high incidence of such mutations among LGMD2A patients ; although it is difficult in the absence of functional assays to differentiate between a polymorphism and a morbid mutation, the occurrence of different "missense" mutations in this gene cannot all be

accounted for as rare private polymorphisms; (2) the failure to observe these mutations in control chromosomes; and (3) the occurrence of mutations in evolutionarily conserved residues and/or in regions of documented functional importance. Four of seven missense mutations change an amino acid which is conserved in all known members of the calpain family in all species (Figure 3). Two of the remaining mutations affect less conserved amino acid residues, but are located in important functional domains. The substitution V354G in exon 8 is 4 residues before the asparagine at the active site and S744G in exon 21 is within the loop of the second EF-hand and may impair the calcium-dependent regulation of calpain activity or the interaction with a small subunit (Figure 4). Several missense mutations change a hydrophobic residue to a polar one, or vice versa (Table 4) possibly disrupting higher order structures.

METHODS

Description of the patients

The LGMD2A families analysed were from 4 different geographic origins. They included 3 Brazilian families, 13 interrelated nuclear families from the Isle of la Réunion, 10 French metropolitan families and 12 US Amish families. The majority of these families were previously ascertained to belong to the chromosome 15 group by linkage analysis (Beckmann, 1991; Young, Passos-Bueno et al., 1993). However, some families from metropolitan France as well as one Brazilian family, B505, had non significant lodscores for chromosome 15. Genomic DNA was obtained from peripheral blood lymphocytes.

Sequencing of cosmid c774G4-1F11 and EcoRI restriction map of cosmids.

Cosmid 1F11 (Figure 1C) was subcloned following DNA preparation through Qiagen procedure (Qiagen Inc., USA) and partial digestion with either *Sau*3A, *Rsa*I or *Alu*I. Size-selected restriction fragments were recovered fom low-melting agarose and eventually ligated with M13 or Bluescript (Stratagene, USA) vectors. After electroporation in *E.coli*, recombinant colonies were picked in 100 µl of LB/ampicillin media. PCR reactions were performed on 1 µl of the culture in 10 mM Tris-HCl, pH 9.0, 50 mM KCl, 1.5 mM MgCl2, 0.1% Triton X-100, 0.01 gelatine, 200µM of each dNTP, 1 U of Taq Polymerase (Amersham) with 100 ng of each vectors primers. Amplification was initiated by 5 min denaturation at 95°C, followed by 30 cycles of 40 sec denaturation at 92°C and 30 sec annealing

at 50°C. PCR products were purified through Microcon devices (Amicon, USA) and sequenced using the dideoxy chain termination method on an ABI sequencer (Applied Biosystems, Foster City, USA). The sequences were analysed and alignments performed using the XBAP software of the Staden package, version 93.9 (Staden, 1982). Gaps between sequence contigs were filled by walking with internal primers. *EcoR*I restriction map of cosmids was performed essentially as described in Sambrook et al. (1989).

Northern Blot analysis

The probes were labelled by random priming with dCTP-($a^{32}P$). Hybridisation was performed to human multiple tissue northern blots as recommended by the manufacturer (Clontech, USA).

Analysis of PCR products from LGMD2A families

One hundred ng of human DNA were used per PCR under the buffer and cycle conditions described in Fougerousse (1994) (annealing temperature shown in Table 3). Heteroduplex analysis (Keene et al., 1991) was performed by electrophoresis of ten µl of PCR products on a 1.5 mm-thick Hydrolink MDE gels (Bioprobe) at 500-600 volt for 12-15 h depending of the fragment length. Migration profile was visualised under UV after ethidium bromide staining.

For sequence analysis, the PCR products were subjected to dye-dideoxy sequencing, after purification through microcon devices (Amicon, USA). When necessary, depending on the nature of the mutations (e.g., frameshift mutation or for some heterozygotes), the PCR products were cloned using the TA cloning kit from Invitrogen (UK). One µl of product was ligated to 25 ng of vector at 12°C overnight. After electroporation into XL1-blue bacteria, several independent clones were analysed by PCR and sequenced as described above.

The invention results from the finding that the nCL1 gene when it is mutated is involved in the etiology of LGMD2A. It is exactly the contrary to what is stated in the litterature, e.g. that the disease is accompanied by the presence of a deregulated calpaïn. Identification of nCL1 as the defective gene in LGMD2A represents the first example of muscular dystrophy caused by mutation affecting a gene which is not a structural component of muscle tissue, in contrast with previously identified muscular dystrophies such as Duchenne and Becker (Bonilla et al., 1988), severe childhood autosomal recessive (Matsumara et al.,

1992), Fukuyama (Matsumara et al., 1993) and merosin-deficient congenital muscular dystrophies (Tomé et al., 1994).

The understanding of the LGMD2A phenotype needs to take into account the fact that there is no active nCL1 protein in several patients, a loss compatible with the recessive manifestation of this disease. Simple models in which this protease would be involved in the degradation or destabilisation of structural components of the cytoskeleton, extracellular matrix or dystrophin complex can therefore be ruled out. Furthermore, there are no signs of such alterations by immunocytogenetic studies on LGMD2 muscle biopsies (Matsumara et al., 1993; Tomé et al., 1994). Likewise, since LGMD2A myofibers are apparently not different from other dystrophic ones, it seems unlikely that this calpain plays a role in myoblast fusion, as proposed for ubiquitous calpains (Wang et al., 1989).

All the data disclosed in these examples confirm that the nCL1 gene is a major gene involved in the disease when mutated.

The fact that morbidity results from the loss of an enzymatic activity raises hopes for novel pharmaco-therapeutic prospects. The availability of transgenic models will be an invaluable tool for these investigations.

The invention is also relative to the use of a nucleic acid or a sequence of nucleic acid of the invention, or to the use of a protein coded by the nucleic acid for the manufacturing of a drug in the prevention or treatment of LGMD2.

The finding that a defective calpain underlies the pathogenesis of LGMD2A may prove useful for the identification of the other loci involved in the LGMDs. Other forms of LGMD may indeed be caused by mutations in genes whose products are the CANP substrates or in genes involved in the regulation of nCL1 expression. Techniques such as the two-hybrid selection system (Fields et al., 1989) could lend themselves to the isolation of the natural protein substrate(s) of this calpain, and thus potentially help to identify other LGMD loci.

The invention also relates to the use of all or a part of the peptidic sequence of the enzyme, or of the enzyme, product of nCL1 gene, for the screening of the ligands of this enzyme, which might be also involved in the etiology and the morbidity of LGMD2

The ligands which might be involved are for example substrate(s), activators or inhibitors of the enzyme.

The nucleic acids of the invention might also be used in a screening method for the determination of the components which may act on the regulation of the gene expression.

A process of screening using either the enzyme or a host recombinant cell, containing the nCL1 gene and expressing the enzyme, is also a part of the invention.

The pharmacological methods, and the use of nucleic acid and peptidic sequences of the invention are very potent applications.

The methods used for such screenings of ligands or regulatory elements are those described for example for the screening of ligands using cloned receptors.

The identification of mutations in the nCL1 gene provides the means for direct prenatal or presymptomatic diagnosis and carrier detection in families in which both mutations have been identified. Gene-based accurate classification of LGMD2A families should prove useful for the differential diagnosis of this disorder.

The invention relates to a method of detection of a predisposition to LGMD2 in a family or a human being, such method comprising the steps of :

- selecting one or more exons or flanking sequences which are sensitive in said family;
- selecting the primers specific for the or these exons or their flanking sequences, a specific example being the PCR primers of Table 3, or an hybrid thereof,
- amplifying the nucleic acid sequence, the substrate for this amplification being the DNA of the human being to be checked for the predisposition, and
- comparing the amplified sequence to the corresponding sequence derived from Figure 2 or Figure 8.

Table 2 indicates the sequences of the introns-exons junctions, and primers comprising in their structure these junctions are also included in the invention.

All other primers suitable for such RNA or DNA amplification may be used in the method of the invention.

In the same way, any suitable amplification method : PCR (for Polymerase Chain Reaction ®) NASBA ® (for Nucleic acid Sequence Based Amplification), or others might be used.

The methods usually used in the detection of one site mutations, like ASO (Allele specific PCR), LCR, or ARMS (Amplification Refactory Mutation System) may be implemented with the specific primers of the invention.

The primers, such as described in Tables 1 and 3, or including junctions of Table 2, or more generally including the flanking sequences of one of the 24 exons are also a part of the invention.

The kit for the detection of a predisposition to LGMD2 by nucleic acid amplification is also in the scope of the invention, such a kit comprises a least PCR primers selected from the group of :

a) in those described in table 1

b) in those described in table 3

c) those including the introns-exons junctions of Table 2.

d) derived from primers defined in a),b) or c).

The nucleic acid sequence of claim 1 to 3 might be inserted in a viral or a retroviral vector, said vector being able to transfect a packaging cell line.

The packaging transfected cell line, might be used as a drug for gene therapy of LGMD2.

The treatment of LGMD2 disease by gene therapy is implemented by a pharmaceutical composition containing a component selected from the group of :

a) a nucleic acid sequence according to claims 1 to 4,

b) a cell line according to claim 24,

c) an aminoacid sequence according to claims 5 to 9.

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CLAIMS

1. A nucleic acid sequence comprising :

1) the sequence represented in Figure 8; or

2) the sequence represented in Figure 2; or

3) a part of the sequence of Figure 2 with the proviso that it is able to code for a protein having a calcium dependant protease activity involved in a LGMD2 disease ; or

4) a sequence derived from a sequence defined in 1), 2) or 3) by substitution, deletion or addition of one or more nucleotides with the proviso that said sequence still codes for said protease.

2. A nucleic acid sequence that is complementary to a nucleic acid sequence according to claim 1.

3. A nucleic acid sequence comprising in its structure a nucleotidic sequence according to claim 1 or 2, under the control of regulatory elements, and involved in the expression of calpaïn activity in a LGMD2 disease.

4. A nucleic acid sequence encoding the aminoacid sequence represented in Figure 2.

5. An amino acid sequence which is coded by a nucleic acid sequence according to claims 1 to 4, characterized in that it is a calcium dependent protease enzyme belonging to the calpaïn family, involved in the etiology of LGMD2.

6. An aminoacid sequence according to claim 5 or 6, characterized in that either it contains the sequence such as represented in Figure 2, or the amino acid sequence of Figure 2 modified by deletion, insertion and/or replacement of one or more amino acids with the proviso that such aminoacid sequence has the calpaïn activity involved in LGMD2 disease.

7. An amino acid sequence according to claim 5 or 6, characterized in that LGMD2 is LGMD2A.

8. A host cell unable to express a calpaïn enzyme activity, characterized in that it is transformed or transfected with a nucleic acid sequence comprising all or part of the nucleic acid sequence according to any one of claims 1 to 4.

9. Use of a nucleic acid according to one of claims 1 to 4 or a host cell according to claim 8 in the manufacturing of a drug for the prevention or the treatment of an LGMD2 disease.

10. Use of an amino acid sequence according to claims 5 to 6 in the manufacturing of a drug for the prevention or the treatment of an LGMD2 disease.

11. Use according to claims 10 or 11, characterized in that LGMD2 is LGMD2A.

12. Use of an amino acid sequence according to claims 5 to 7 for the screening of the ligands of said amino acid sequence, said ligand being selected in a group consisting of substrate(s), co-factors or regulatory components.

13. Use of a nucleic acid sequence according to one of claims 1 to 4 in a screening method for the determination of the components which may act on the regulation of gene expression of calpaïn.

14. Use of an host cell according to claim 8 in a screening method for the determination of components active on the expression of the calpaïn.

15. A method for detecting of a predisposition to a LGMD2 disease in a family or a human being, such method comprising the steps of :

- selecting one or more exons or their flanking sequences of the gene,
- selecting primers specific for these exons, or their flanking sequences, or an hybrid thereof,
- amplifying the nucleic acid sequences with these primers, the substrate for this amplification being the DNA of a human being; and
- comparing the amplified sequence to the corresponding sequence derived from Figure 2 or Figure 8.

16. The method according to claim 15, characterized in that the primers are those selected from the group of :

a) those described in Table 1;

b) those described in Table 3; and

c) those including the introns-exons junctions of Table 2;

d) those derived from the primers in a), b), or c).

17. The method according to claim 15 or 16, characterized in that LGMD2 is LGMD2A.

18. A kit for the detection of a predisposition to LGMD2 by nucleic and amplification characterized in that it comprises primers selected from the group of :

a) those described in Table 1;

b) those described in Table 3; and

c) those including the introns-exons junctions of Table 2;

d) those derived from the primers in a), b) or c).

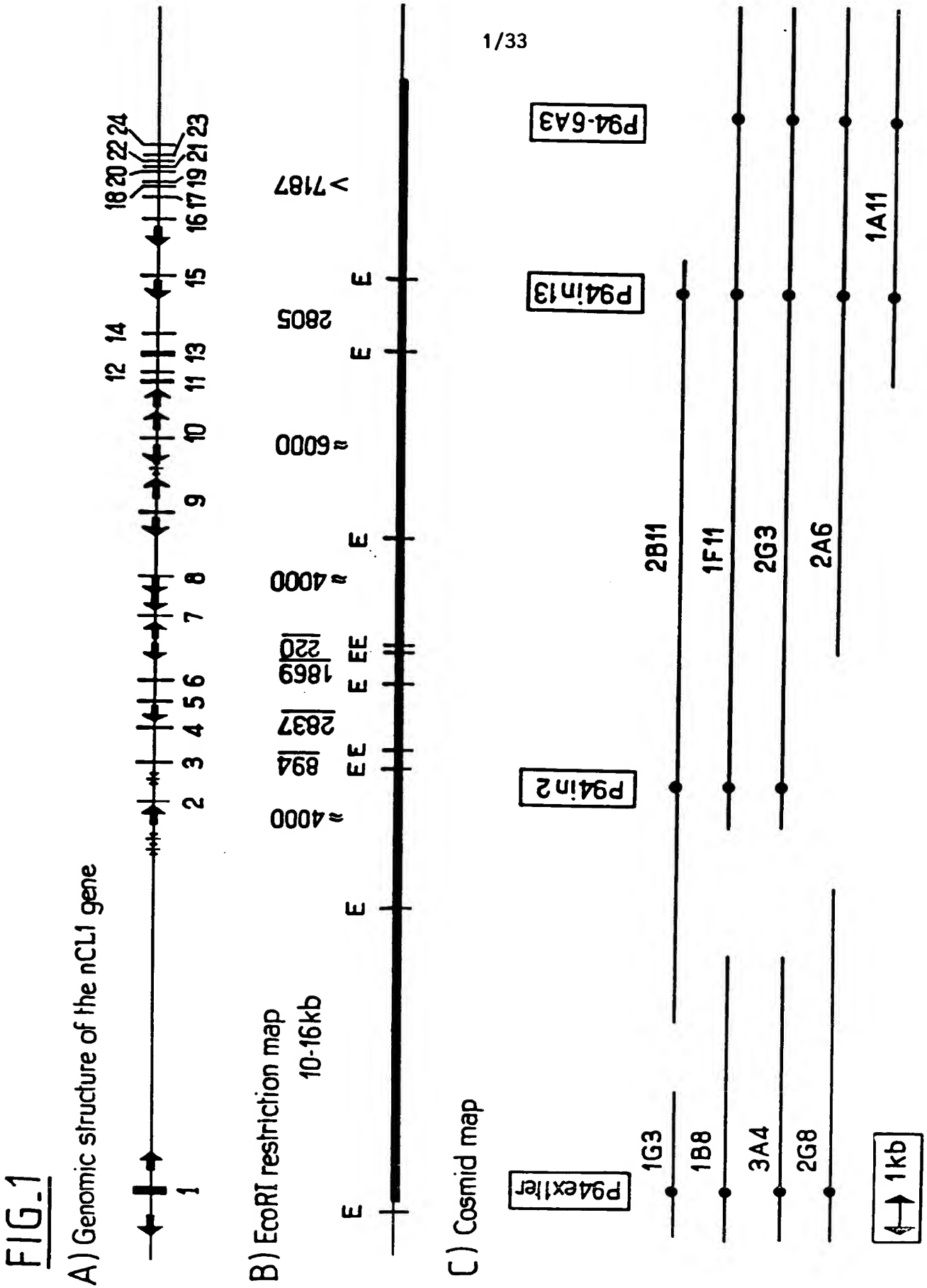
19. Use of a host cell according to claim 8 in a manufacturing of a drug for gene therapy of an LGMD2 disease.

20. Pharmaceutical composition for the treatment of an LGMD2 disease characterized in that in contains a component selected from the group of :

a) a nucleic acid sequence according to claims 1 to 4,

b) a host cell according to claim 8,

c) an aminoacid sequence according to claims 5 to 7.


A) Genomic structure of the nCL1 gene
1
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
B) EcoRI restriction map
10-16kb
E
≈4000
894
2837
1869
220
E E E E E
≈4000
E
2805
E
E
>7187
C) Cosmid map
P94ex1ter
P94in2
P94in13
P94-6A3
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3A4
2G8
2B11
1F11
2G3
2A6
1A11
1kb

FIG_1

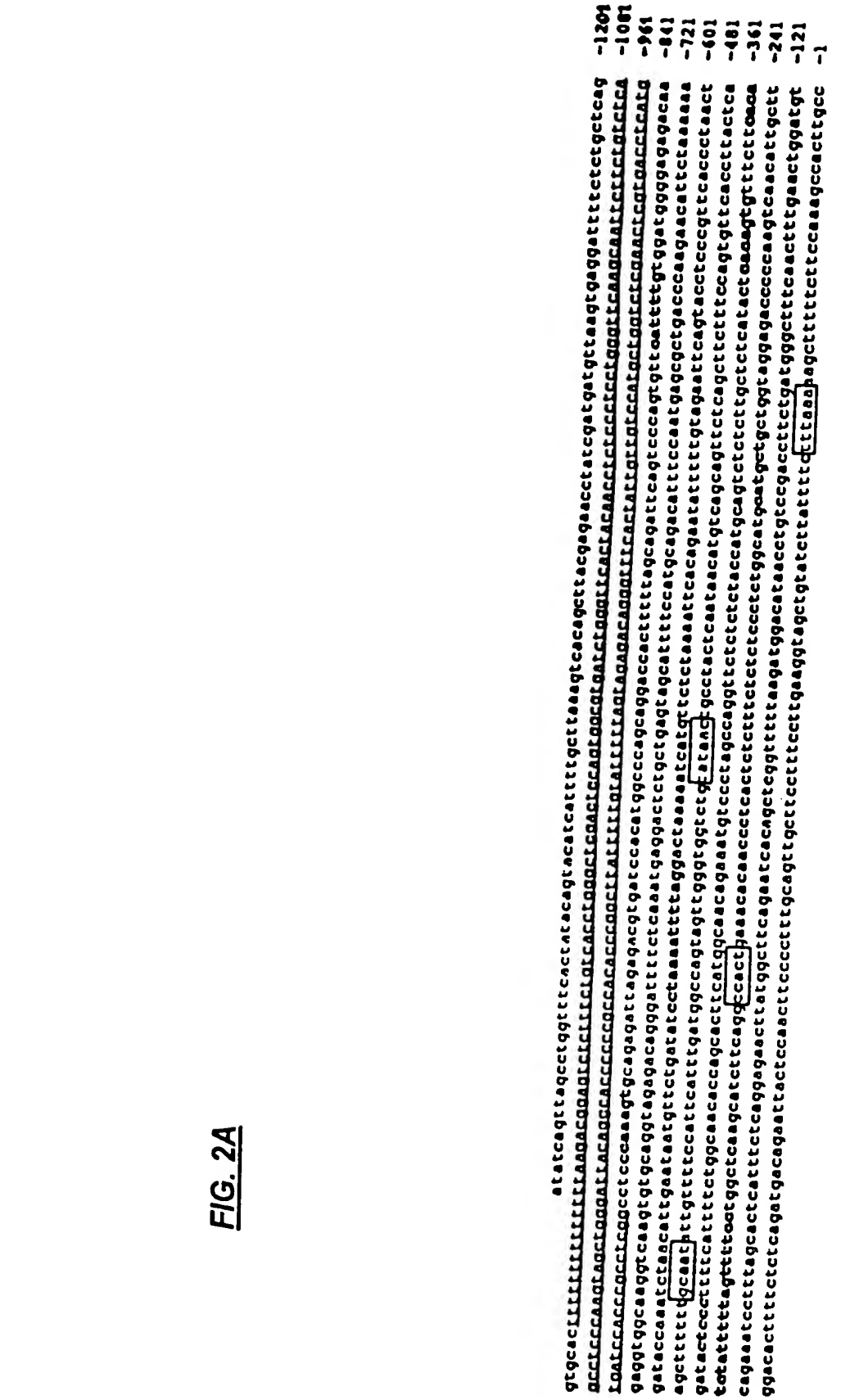


FIG. 2A

FIG. 2B/1

1 ATGCCGACCGTCATTAGCGCATCTGTGGCTCCAAGGACAGCGGCTGAGCCCCGGTCCCCAGGGCCAGTTCCTCACCCGGCCCAGAGCAAGGCCACTGAGGCTGGGGGTGGAAACCCAAGT
M P T V I S A S V A P R T A A E P R S P G P V P H P A Q S K A T E A G G G N P S

121 GGCATCTATTCAGCCATCATCAGCCGCAATTTTCCTATTATCGGAGTGAAAGAGAAGACATTCGAGCAACTTCACAAGAAATGTCTAGAAAAGAAAGTTCTTTATGTGGACCCTGAGTTC
G I Y S A I I S R N F P I I G V K E K T F E Q L H K K C L E K K V L Y V D P E F

241 CCACCGGATGAGACCTCTCTCTTTTATAGCCAGAAGTTCCCCATCCAGTTCGTCTGGAAGAGACCTCCGGAAATTTGCGAGAATCCCCGATTTATCATTGATGGAGCCAACAGAACTGAC
P P D E T S L F Y S Q K F P I Q F V W K R P P E I C E N P R F I I D G A N R T D

361 ATCTGTCAAGGAGAGCTAGGGGACTGCTGGTTTCTCGCAGCCATTGCCTGCCTGACCCTGAACCAGCACCTTCTTTTCCGAGTCATACCCCATGATCAAAGTTTCATCGAAAACTACGCA
I C Q G E L G D C W F L A A I A C L T L N Q H L L F R V I P H D Q S F I E N Y A

481 GGGATCTTCCACTTCCAGTTCTGGCGCTATGGAGAGTGGGTGGACGTGGTTATAGATGACTGCCTGCCAACGTACAACAATCAACTGGTTTTCACCAAGTCCAACCACCGCAATGAGTTC
G I F H F Q F W R Y G E W V D V V I D D C L P T Y N N Q L V F T K S N H R N E F

601 TGGAGTGCTCTGCTGGAGAAGGCTTATGCTAAGCTCCATGGTTCCTACGAAGCTCTGAAAGGTGGGAACACCACAGAGGCCATGGAGGACTTCACAGGAGGGGTGGCAGAGTTTTTTGAG
W S A L L E K A Y A K L H G S Y E A L K G G N T T E A M E D F T G G V A E F F E

721 ATCAGGGATGCTCCTAGTGACATGTACAAGATCATGAAGAAAGCCATCGAGAGAGGCTCCCTCATGGGCTGCTCCATTGATGATGGCACGAACATGACCTATGGAACCTCTCCTTCTGGT
I R D A P S D M Y K I M K K A I E R G S L M G C S I D D G T N M T Y G T S P S G

841 CTGAACATGGGGGAGTTGATTGCACGGATGGTAAGGAATATGGATAACTCACTGCTCCAGGACTCAGACCTCGACCCCAGAGGCTCAGATGAAAGACCGACCCGGACAATCATTCCGGTT
L N M G E L I A R M V R N M D N S L L Q D S D L D P R G S D E R P T R T I I P V

961 CAGTATGAGACAAGAATGGCCTGCGGGCTGGTCAGAGGTCACGCCTACTCTGTCACGGGGCTGGATGAGGTCCCGTTCAAAGGTGAGAAAGTGAAGCTGGTGCGGCTGCGGAATCCGTGG
Q Y E T R M A C G L V R G H A Y S V T G L D E V P F K G E K V K L V R L R N P W

1081 GGCCAGGTGGAGTGGAACGGTTCTTGGAGTGATAGATGGAAGGACTGGAGCTTTGTGGACAAAGATGAGAAGGCCCGTCTGCAGCACCAGGTCACTGAGGATGGAGAGTTCTGGATGTCC
G Q V E W N G S W S D R W K D W S F V D K D E K A R L Q H Q V T E D G E F W M S

1201 TATGAGGATTTCATCTACCATTTCACAAAGTTGGAGATCTGCAACCTCACGGCCGATGCTCTGCAGTCTGACAAGCTTCAGACCTGGACAGTGTCTGTGAACGAGGGCCGCTGGGTACGG
Y E D F I Y H F T K L E I C N L T A D A L Q S D K L Q T W T V S V N E G R W V R

FIG. 2B/2

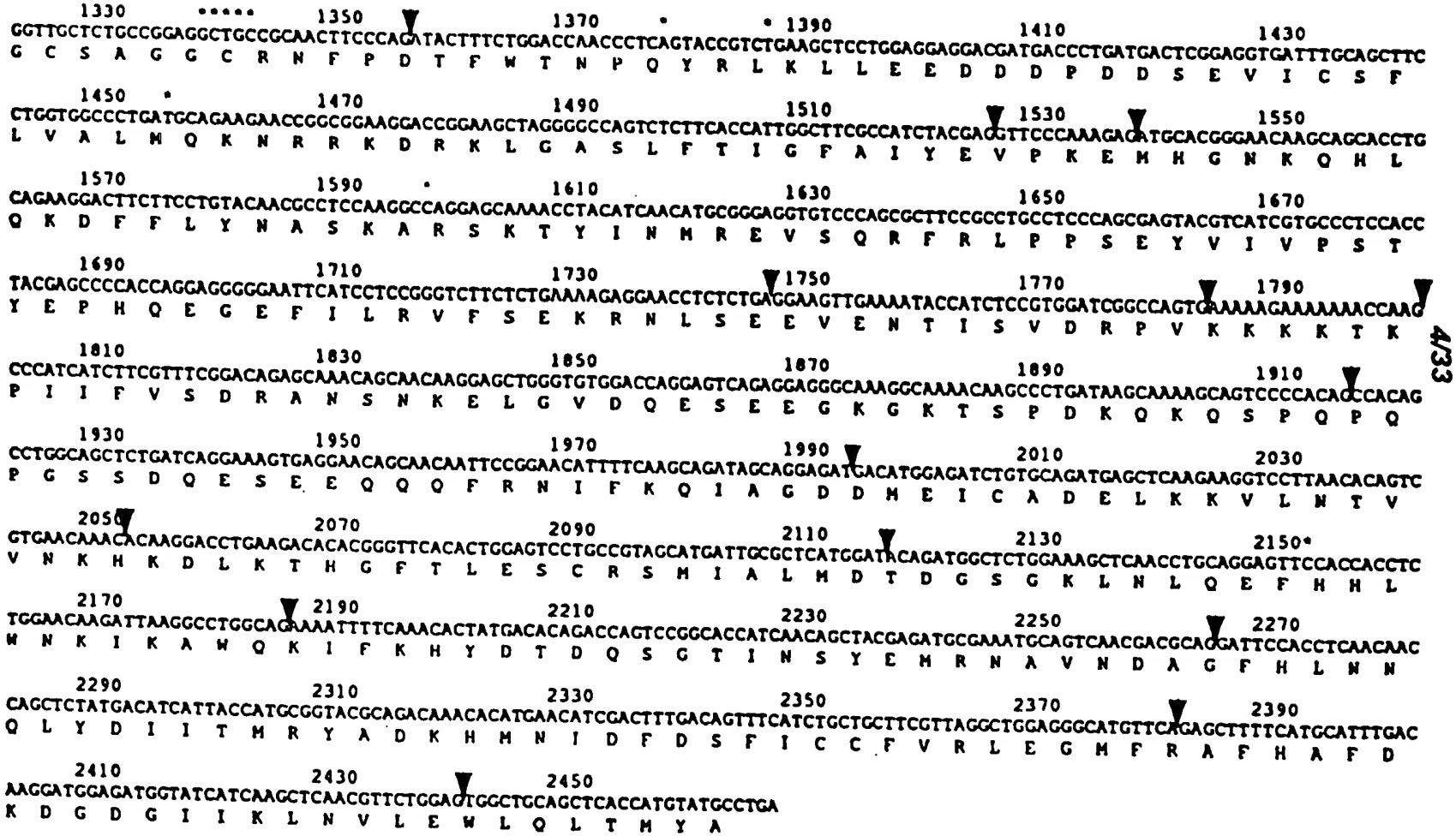

1330 1350 1370 1390 1410 1430
GGTTGCTCTGCCGGAGGCTGCCGCAACTTCCCAGATACTTTCTGGACCAACCCTCAGTACCGTCTGAAGCTCCTGGAGGAGGACGATGACCCTGATGACTCGGAGGTGATTTGCAGCTTC
G C S A G G C R N F P D T F W T N P Q Y R L K L L E E D D D P D D S E V I C S F
1450 1470 1490 1510 1530 1550
CTGGTGGCCCTGATGCAGAAGAACCGGCGGAAGGACCGGAAGCTAGGGGCCAGTCTCTTCACCATTGGCTTCGCCATCTACGAGGTTCCCAAAGAGATGCACGGGAACAAGCAGCACCTG
L V A L M Q K N R R K D R K L G A S L F T I G F A I Y E V P K E M H G N K Q H L
1570 1590 1610 1630 1650 1670
CAGAAGGACTTCTTCCTGTACAACGCCTCCAAGGCCAGGAGCAAAACCTACATCAACATGCGGGAGGTGTCCCAGCGCTTCCGCCTGCCTCCCAGCGAGTACGTCATCGTGCCCTCCACC
Q K D F F L Y N A S K A R S K T Y I N M R E V S Q R F R L P P S E Y V I V P S T
1690 1710 1730 1750 1770 1790
TACGAGCCCCACCAGGAGGGGGAATTCATCCTCCGGGTCTTCTCTGAAAAGAGGAACCTCTCTGAGGAAGTTGAAAATACCATCTCCGTGGATCGGCCAGTGAAAAAGAAAAAAACCAAG
Y E P H Q E G E F I L R V F S E K R N L S E E V E N T I S V D R P V K K K K T K
1810 1830 1850 1870 1890 1910
CCCATCATCTTCGTTTCGGACAGAGCAAACAGCAACAAGGAGCTGGGTGTGGACCAGGAGTCAGAGGAGGGCAAAGGCAAAACAAGCCCTGATAAGCAAAAGCAGTCCCCACAGCCACAG
P I I F V S D R A N S N K E L G V D Q E S E E G K G K T S P D K Q K Q S P Q P Q
1930 1950 1970 1990 2010 2030
CCTGGCAGCTCTGATCAGGAAAGTGAGGAACAGCAACAATTCCGGAACATTTTCAAGCAGATAGCAGGAGATGACATGGAGATCTGTGCAGATGAGCTCAAGAAGGTCCTTAACACAGTC
P G S S D Q E S E E Q Q Q F R N I F K Q I A G D D M E I C A D E L K K V L N T V
2050 2070 2090 2110 2130 2150
GTGAACAAACACAAGGACCTGAAGACACACGGGTTCACACTGGAGTCCTGCCGTAGCATGATTGCGCTCATGGATACAGATGGCTCTGGAAAGCTCAACCTGCAGGAGTTCCACCACCTC
V N K H K D L K T H G F T L E S C R S M I A L M D T D G S G K L N L Q E F H H L
2170 2190 2210 2230 2250 2270
TGGAACAAGATTAAGGCCTGGCAGAAAATTTTCAAACACTATGACACAGACCAGTCCGGCACCATCAACAGCTACGAGATGCGAAATGCAGTCAACGACGCAGATTCCACCTCAACAAC
W N K I K A W Q K I F K H Y D T D Q S G T I N S Y E M R N A V N D A G F H L N N
2290 2310 2330 2350 2370 2390
CAGCTCTATGACATCATTACCATGCGGTACGCAGACAAACACATGAACATCGACTTTGACAGTTTCATCTGCTGCTTCGTTAGGCTGGAGGGCATGTTCAGAGCTTTTCATGCATTTGAC
Q L Y D I I T M R Y A D K H M N I D F D S F I C C F V R L E G M F R A F H A F D
2410 2430 2450
AAGGATGGAGATGGTATCATCAAGCTCAACGTTCTGGAGTGGCTGCAGCTCACCATGTATGCCTGA
K D G D G I I K L N V L E W L Q L T M Y A

FIG. 2C

SUBSTITUTE SHEET (RULE 26)

Figure 3:

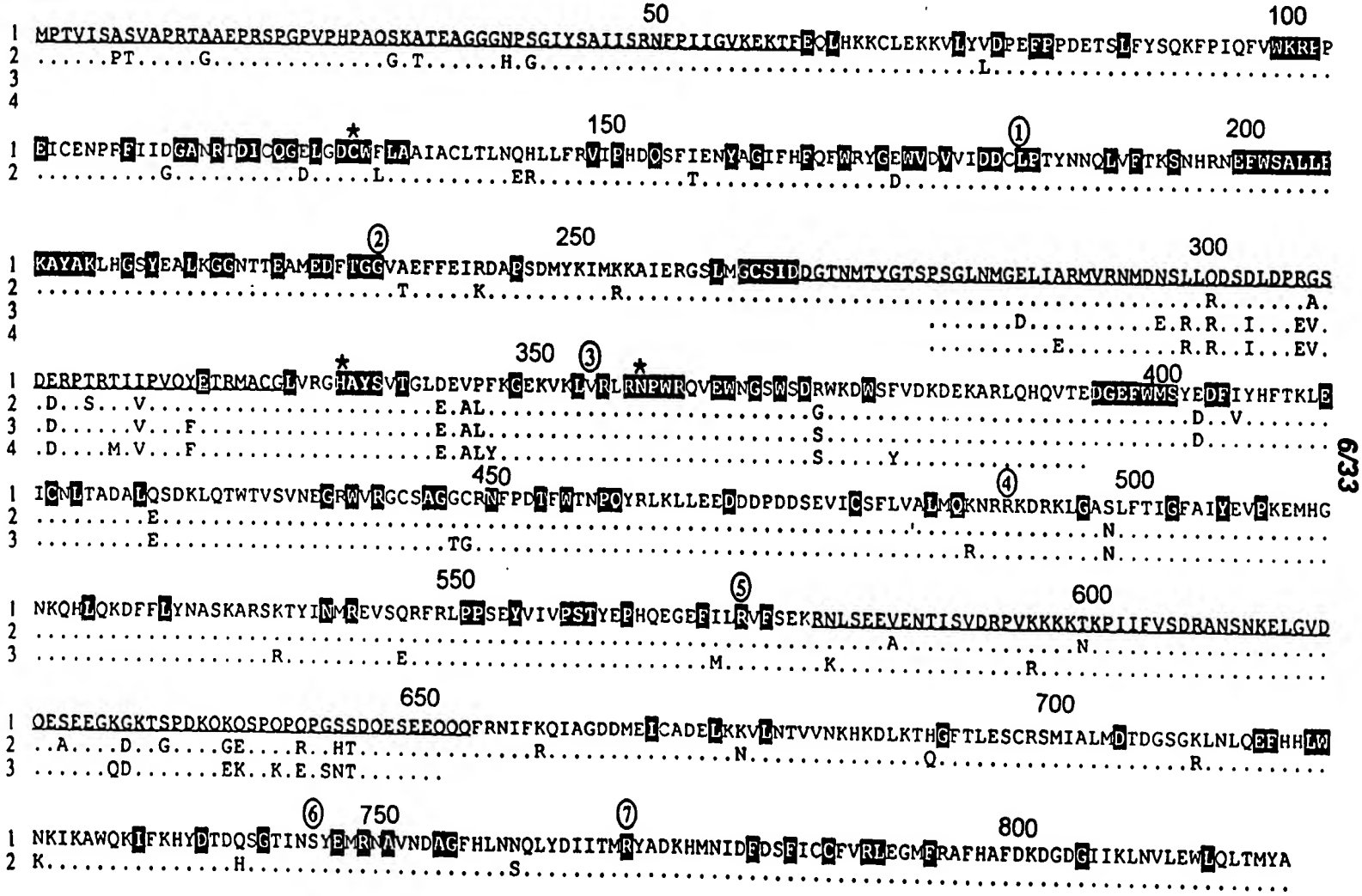
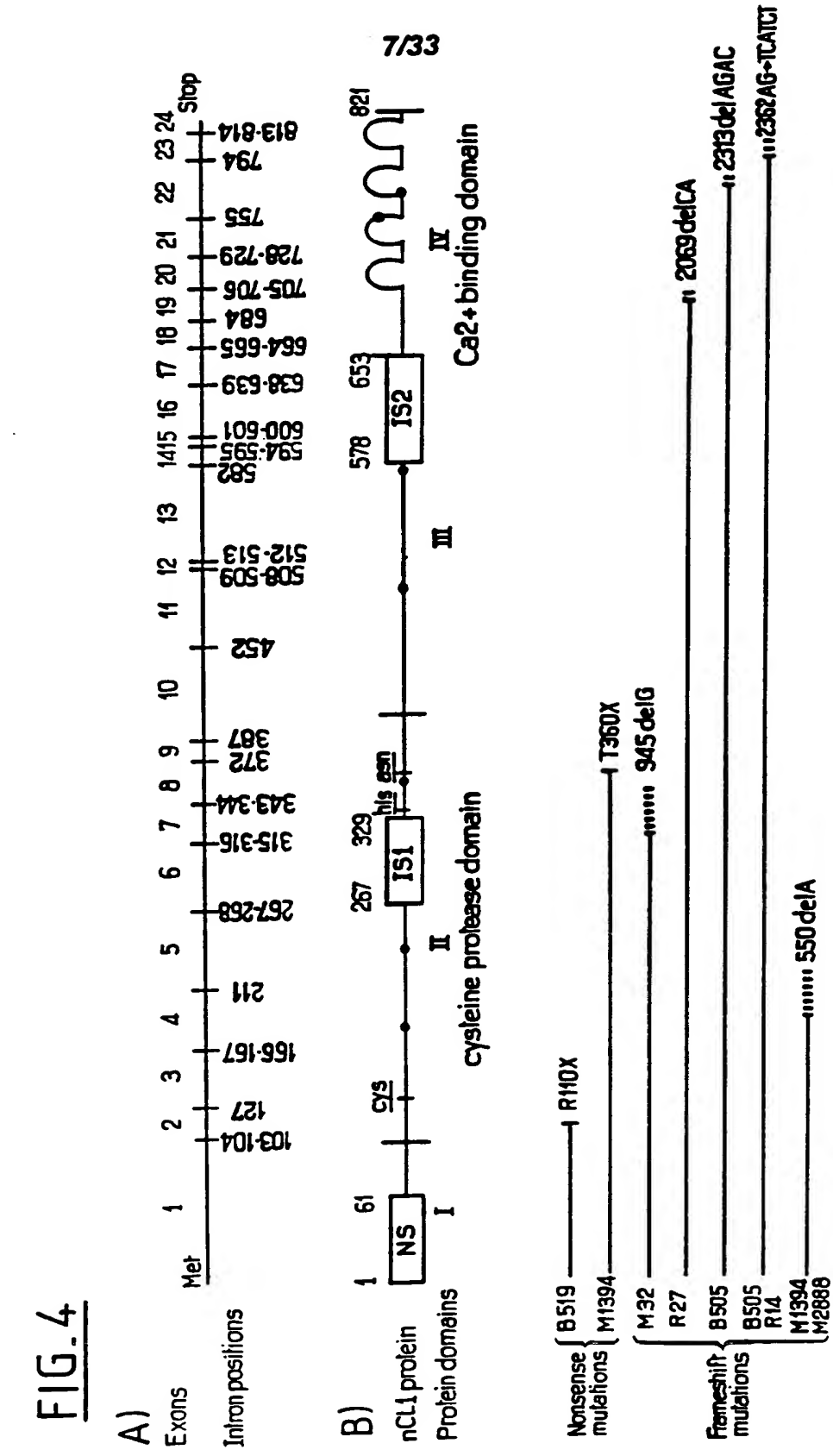
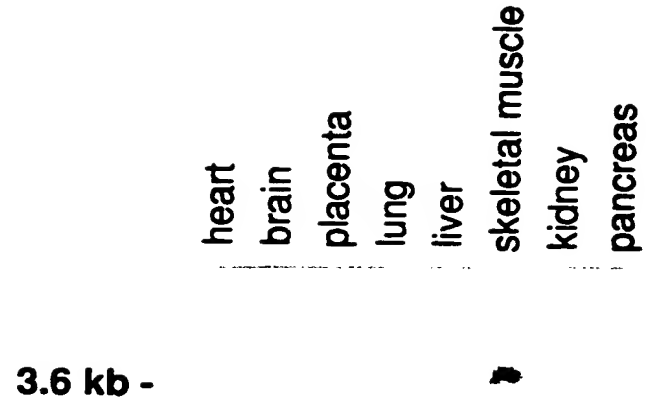


FIG. 4


A)
Exons
Intron positions
Met
Stop
B)
nCL1 protein
Protein domains
NS
IS1
IS2
cysteine protease domain
Ca2+ binding domain
Nonsense mutations
Frameshift mutations
B519
M1394
M32
R27
B505
B505
R14
M1394
M2888
R110X
T360X
945 delG
2069 delCA
2313 del AGAC
2362 AG→TCATCT
550 delA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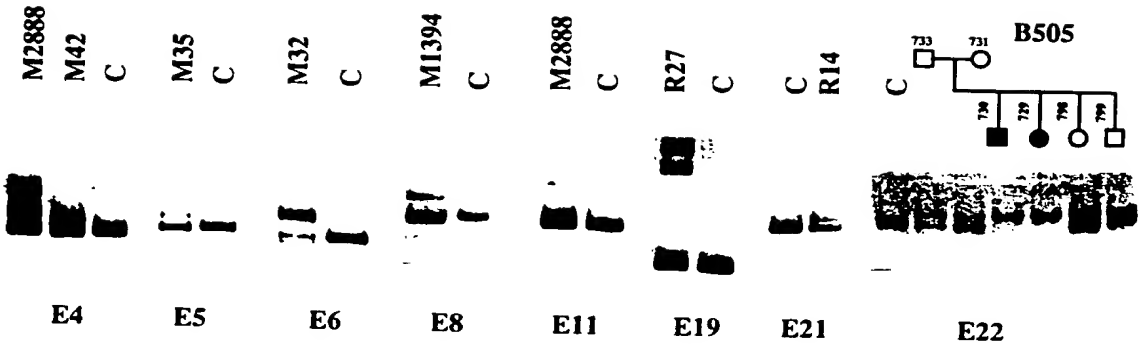
brain
placenta
lung
liver
skeletal muscle
kidney
pancreas
3.6 kb -

FIG. 5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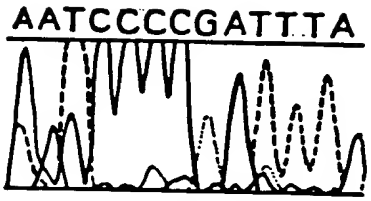
M42
C
M35
C
M32
C
M1394
C
M2888
C
R27
C
C
R14
C
B505
733
731
E4
E5
E6
E8
E11
E19
E21
E22

FIG. 6

FIG.7

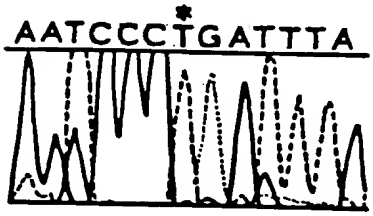
A) EXON 2

Normal sequence



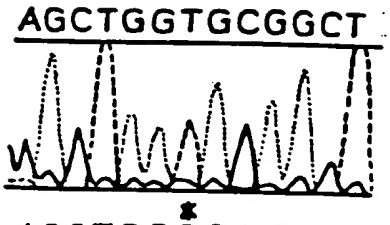
B519

CGA -> TGA
Arg110 Stop



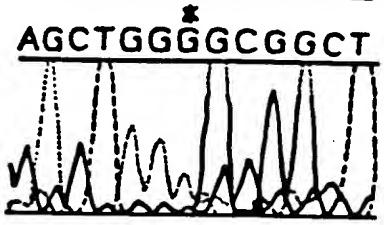
B) EXON 8

Normal sequence



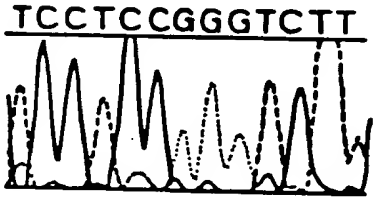
M2407

GTG -> GGG
Val354 Gly



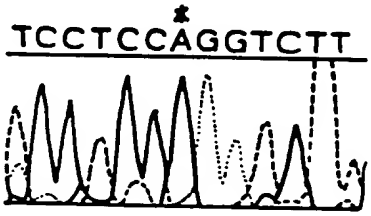
C) EXON 13

Normal sequence



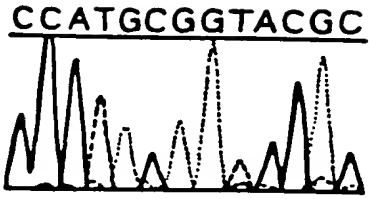
R 12

CGG -> CAG
Arg 572 Gln



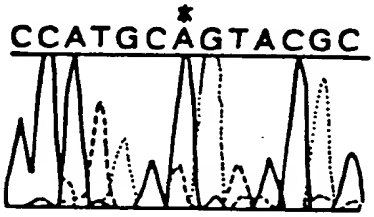
D) EXON 22

Normal sequence



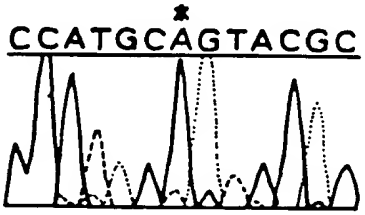
Amish

CGG -> CAG
Arg769 Gln



B501

CGG -> CAG
Arg769 Gln



LISTE DE SEQUENCES

(1) INFORMATION GENERALE:

(i) DEPOSANT:
(A) NOM: AFM
(B) RUE: 13, place de Rungis
(C) VILLE: PARIS
(E) PAYS: FRANCE
(F) CODE POSTAL: 75013
(G) TELEPHONE: (1) 45 65 13 00

(ii) TITRE DE L' INVENTION: LGMD GENE

(iii) NOMBRE DE SEQUENCES: 4

(iv) FORME LISIBLE PAR ORDINATEUR:
(A) TYPE DE SUPPORT: Floppy disk
(B) ORDINATEUR: IBM PC compatible
(C) SYSTEME D' EXPLOITATION: PC-DOS/MS-DOS
(D) LOGICIEL: PatentIn Release #1.0, Version #1.25 (OEB)

(2) INFORMATION POUR LA SEQ ID NO: 1:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 3018 paires de bases
(B) TYPE: acide nucléique
(C) NOMBRE DE BRINS: double
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 1:

TGATAGGTGC TTGTAAACTG TGCTTAACGA AAACATACCG TGTGCTGTAG GGACTTAACT 60

CTTGTTTATA TCAGTTAGCC TGGTTTCGCT AACAGTACAT CATTTTGCTT AAAGTCACAG 120

CTTACGAGAA CCTATCGATG ATGTTAAGTG AGGATTTTCT CTGCTCAGGT GCACTTTTTT 180

TTTTTTTTAA GACGGAGTCT CTTTCTGTCA CCTGGGCTGG AGTGCAGTGG CGTGATCTGG 240

GTTCACAACA ACCTCTGCCT CCTGGGTTCA AGCAATTCTT CTGTCTCAGC CTCCCAAGTA 300

GCTGGGATTA CAGGCACCCG CCGCCACACC CGGCTTATTT TTGTATTTTT AGTAGAGACA 360

GGGTTTCACT ATTGTTGACC ATGCTGGTCT CGAACTCGTG ACCTCATGTG ATCCACCCGC 420

CTCGGCCTCC CAAAGTGCAG AGATTAGAGA CGTGAGCCAC ATGGCCCAGC AGGACCACTT 480

FIG 8A/1

TTTAGCAGAT TCAGTCCCAG TGTTCATTTT GTGGATGGGG AGAGACAAGA GGTGCAAGGT 540

CAAGTGTGCA GGTAGAGACA GGGATTTTCT CAAATGAGGA CTCTGCTGAG TAGCATTTTC 600

CATGCAGACA TTTCCAATGA GCGCTGACCC AAGAACATTC TAAAAAGATA CCAAATCTAA 660

CATTGAATAA TGTTCTGATA TCCTAAAATT TTAGGACTAA AAATCATGTT CTCTAAAATT 720

CACAGAATAT TTTTGTAGAA TTCAGTACCT CCCGTTCACC CTAACTAGCT TTTTTGCAAT 780

ATTGTTTTCC ATTCATTTGA TGGGCAGTAG TTGGGTGGTC TGTATAACTG CCTACTCAAT 840

AACATGTCAG CAGTTCTCAG CTTCTTTCCA GTGTTCACCT TACTCAGATA CTCCCTTTTC 900

ATTTTCTGTC AACACCAGCA CTTCATGTCA ACAGAAATGT CCCTAGCCAG GTTCTCTCTC 960

TACCATGCAG TCTCTCTTGC TCTCATACTC ACAGTGTTTC TTCACATCTA TTTTTAGTTT 1020

TCCTGGCTCA AGCATCTTCA GGCCACTGAA ACACAACCCT CACTCTCTTT CTCTCTCCCT 1080

CTGGCATGCA TGCTGCTGGT AGGAGACCCC CAAGTCAACA TTGCTTCAGA AATCCTTTAG 1140

CACTCATTTC TCAGGAGAAC TTATGGCTTC AGAATCACAG CTCGGTTTTT AAGATGGACA 1200

TAACCTGTCC GACCTTCTGA TGGGCTTTCA ACTTTGAACT GGATGTGGAC ACTTTTCTCT 1260

CAGATGACAG AATTACTCCA ACTTCCCCTT TGCAGTTGCT TCCTTTCCTT GAAGGTAGCT 1320

GTATCTTATT TTCTTTAAAA AGCTTTTTCT TCCAAAGCCA CTTGCCATGC CGACCGTCAT 1380

TAGCGCATCT GTGGCTCCAA GGACAGCGGC TGAGCCCCGG TCCCCAGGGC CAGTTCCTCA 1440

CCCGGCCCAG AGCAAGGCCA CTGAGGCTGG GGGTGGAAAC CCAAGTGGCA TCTATTCAGC 1500

CATCATCAGC CGCAATTTTC CTATTATCGG AGTGAAAGAG AAGACATTCG AGCAACTTCA 1560

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CGGCAGCTCA GCTGTGCACA TGGGCACTGG GGGAAGGATC CTGGCAGCAG CTCTGCTGGG 1800

CTCTGTCTTT AAGTGTGAAG CAGGGAGGAG AGGAACAGGT CTCAGATATT TCACCAAATC 1860

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TCTCTGAAAA AAAAAAAAAA ATCTTGCTTT TTATAAAAGT GGGTGGAACT CAGTTTAATT 1980

CATCCTGTAA AAATAAATAT TCCTTTCTCA GAACAAATTC CAGACAGCCC AGATGTACCT 2040

GTTCGTTTTA ATATTATTCA TCTTGGTAAG ATTATTTCAG TTTCTCTGGC TAAAATCATG 2100

FIG 8A/2

ATGTTATTCT TCTTTAATTT ACCAATGGCC ATTCTTTCTG AAACACAGAA ACCCTAGAAA 2160

GAGAAGAGTC ATAGGCAAGG AATTTTTTTC ATGCATAAAA TGTTGGGGTT AAAGAGAGAG 2220

AGACCTAGCA ATCGCTTTGG TCCACCTACC TCACCTCATA AGTGAGGAGT CAAGGCACAC 2280

TAGAGTGAAA TATATCTAGT GGGCACATGA CAGAGCCCGG ATTAAAACTT TGTTTTAGGA 2340

AACTCTCCCA GCCTCTGGGT TTCATTTACA GTGATCGCCA GGAGGGAAAT CACATTCCCC 2400

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AAGAGTGACA TTGTCAGGAG ACGATGTTCT GGGTGCCACA GGATCATGTG GCAAGGAGAG 2640

CTAACCTGGT CCAGGGAGAC AAACCCTCTC TGAGGAAATG ATGACAAGCT GAGACCCAAT 2700

ACTATTGATT AGCCATGGTT TTCTTTAACC TAAGGTGGGC CAGGCATGGT GGCTCATGCC 2760

TATAAACCCA GCATTTTGGA AGGCCCAGGC TGGAGGATTG CTTGAGCCCA AGAGTTAGAG 2820

ACCAGCCTGG GCAACAGGGT GAAAACCTAT CTCTTTTGTA CTAAAAATTC AAAAAATTAT 2880

CCAGGCATGG TGGCACATGC CTGTGGTCCT AGCTACTCAG AGGCTGAGGT GGGAAGATCA 2940

CTTGAACTCG GGGAGTTTGA GGCAGCAGTG AGCCGAGATC ATGCCACTGC ACTCCAGGCT 3000

GGGTGACAGG AGTGAGAC 3018

(2) INFORMATION POUR LA SEQ ID NO: 2:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 11451 paires de bases
(B) TYPE: acide nucléique
(C) NOMBRE DE BRINS: double
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 2:

GATCCACCCG CCTTGGCCTC CCAAAGTGCT GAGATTACAG GTGTGAGCCA CCACGCCCAG 60

CCGACACTGC CCTAACTCTC AAGTTGCATC CTTACTCGAA TAGTATGACA GTGTGGGAAG 120

CAGCATGGGA CAATGTAAAA AGGAGGCATG TTTCTGGCTT CTGCTACTTA CTAGCTGTGT 180

GTCTTTGCAC GAGTTTCTTA ACCTCTCTGG GCCTCAGTTT CCTTATCTGA AAAATAACAA 240

TGATAGTATT CCCTTCACAG GGCCAAATGG AATACTATCA GGAACACTAC ATAATGGAAC 300

TCAATAAATA ATAGCTACTG CGGCCGGGCG CGGTGGCTCA CATCTGTAAT CCCAGCACTT 360

TGGGAGGCCG AGGCGGGTGG ATCACAAGGT CAAGAGATGG AGACCATCCT GGCCAACATG 420

GTGAAACCGT ATCTCTACTA AAGATACAAA AATTAGCTGG GCATGGTGGC GCATGCCTAT 480

AGTCCCAGCT ACTCGAGAGG CTGAGGCAGG AGAATCACTT GAACCCCGGA GGCAGAGGTT 540

TCAGTGAGCC AAGATTGCAC CAGTGCACTG CAGCCTGGCG ACAGAGTGAG ACTCCGTCTC 600

AAAAAAATAC CTATCTATCT ATCTGTCTAT CTACTGTTAT TCTTACCTGG TCATTTCCTT 660

TTTGTTTCAC AGGAAATTTG CGAGAATCCC CGATTTATCA TTGATGGAGC CAACAGAACT 720

GACATCTGTC AAGGAGAGCT AGGTAGGAAA GTGCCTCAGG TCAGATCCTG CCAGATGATC 780

AAGGGGTGAT TACAAGGTGT GATCCCCTTC CAGGAGGTAA AGGGACAATC TGTGCTTGCT 840

TCCAGTAACT TTTTGGAAGA TTTTTTATAA CAGTTGCTTT ATGGTCGTTT ATCTACATGC 900

TGGCGATTGC TTCATTTCCT CCTACATGCC TCTTTAGCAC TCTGCCATGC ATCACAGGGG 960

GTATCTGCAT CCTGTGGCCT CCTCTCCAGT ATCTCAAGGA CACTTACATA CCCCACTCAG 1020

CATGACAAAA GCCCTGCTTT TCACTGTATC GTCTTTCTTG GAAGACAGCT CTGTGACTGT 1080

GCACCAAGCA TGCCCCTTGG GCATGGAGAT TCTAGATACA CACACAAAAG GCATCGCCAA 1140

GGAAAGCACT TGTAACTGGA ACCCTTGGTT TAAATTGGCC CAGCATAGCT CCATCTTTAA 1200

FIG. 8/B I

AAGAGTCTTT CCACAAAGAT GGCATCCGCC ATGTGGATGA GCATCCAATT TTCTCTTTGA 1260

TTGGTTAGCT TGACTGCTCC ATCTGATCTT CCTCTCTCTC GACCTCTTGT TCAGAAAGTA 1320

TTGTCTTTGG TGTGGACTAT AAGCAAGCTC TGTGAAGTAA AATTGGAGAG AACACCAACA 1380

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GAAGGCAGTT CTTTGTGACC AGGGTACACC CCCTATTATA TATATATATA CACACACAGA 1740

GAGAGAGAGA GAGAGAGAGA GAGAGAAAGA GAGCAAAGTG TTACCTCCAA CTACATACAG 1800

TACTCTGTCA GAAAAGAGGT TCAGAGAATA AGAAAACGTC CCGAGCTCAT TCCGTTGCCA 1860

GCAATGTCTT ACTGCCCCCT ATAGACGGGT TCCAGGGCAG CTGCCTACCT GGCCTTCCTT 1920

CCAATACAAA TCATCTTGGT GGATGGTTCT CTGAGGCTCA GTCTTCGCTG AAGTCAGAAG 1980

AGGAATTGGA CTCACATTGC AAAGGCACAG GGCAGGGCAG ATTTCCTACA GGTGTTAGGA 2040

AGAACAACCC AGTTATGATC ACCTACTGCT CTGTCTCCAT TGAGGCCTAA AAAGGAAGTG 2100

AGTTTATACT GCAGTTGGAG GAACTGCCTG CAGCCTTGAG GAAAATGTCT AGTCACAAGG 2160

GAGTAAGTTA CCTGTTGATC ATATTGTCAA GGAATTCCTG TCCAATTCTC CTTCCCTGGG 2220

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TCAGGGAAGT GGAGTGGCTG GCTGGGATTG GGGCTTTTTC TTCCCAGGAG GAGCAGGAGT 2340

GCTCACGATC TGTGCCCTGT GTCTGCCTGC AGGGGACTGC TGGTTTCTCG CAGCCATTGC 2400

CTGCCTGACC CTGAACCAGC ACCTTCTTTT CCGAGTCATA CCCCATGATC AAAGTTTCAT 2460

CGAAAACTAC GCAGGGATCT TCCACTTCCA GGTGAGGTAA TGAGAGTGTA GTTAAGAGGG 2520

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GTCTGGCTGC TTTTATTGCC TGCAGCCCTT CTCAAGTAGG TCCCTAAGAT ATTAGCACTG 2820

FIG. 8B/2

TGACACCACA GGACCCTTCA GGTTGTACAG GAACCCCTGT CCAGGGCTCC TGTATACTTC 2880

TTCCTCTCTA AGGCATGGCG GTACCAAGGC TATCACTCCT CTCTTCCAAG CCCTGGAAGA 2940

AGAGTCTGCT TAACCTGGGG ATCAGGCTTC TTGTTTGCCC TAGAACTGAA TCTGATGGTT 3000

CTAGAATCCA TCCAGCTACT GGAAATTTTC TGGGTCCCAG TCACCTTGGC ATAGAGCTGG 3060

TGCTAGAGCA GAACCAAACT GAATTCTACC TGTGAGGGTC TCGTAGCTTC CGGGATGCTG 3120

GGGAGTCAGC CTGTCTCCAG CTTCAAAGGC TCCCTCATGT CCCAGGATGA CCCACATTAT 3180

CAGTTCTTGC TCCCCGGGTC TTGCACCTCA GCACGGAAGG CCTCAGAAAA GGTCTGTCTC 3240

CAGGCTCAGA CTCCCCCTCC TGCCGCCTTG GGAACATGGC ATATTTAAAG GGTCTCAGAT 3300

CTAAAGGGCC TTACATACAA ATATCAGATA GATTTCTGTT CTCATTTCAA TGAGGGAGAA 3360

AGTGCCATTG AAAAGGAGAC TAAACCACAT TTGGCCCTTT TCAGTTCAAA CTGATTCATT 3420

CAAAAAAGAG CGACATCCAA ACTTGAAATG ATTGAACAAT GTTCCTGCTA CAGCTAGAAT 3480

AGATTCTGGG TCACTTTGTT CCTCCGTTTC AATCCTTGTT CTTCAGTTTG GCATCAAGAA 3540

ATACCTAAAT CAGCACAGTG CCTTCACTGC ATAGTTCCCA ATCCTGGCCA CATTGAATCA 3600

GCTGGGGGCA CCTGAGAGTG CTGACACCCA GGCCCTGCCC CAGACCTGCT GAGCAGGAGA 3660

ATGAAAATCT TACATCCTAA GACACTCATG GAGCACCTAC TCTACCCATT ACTGGGCTGG 3720

ACTCTGTGGA AGACATGAAG TATATGTAAC TCACTTCCAG CTCTCAAAAA GCACCCAGTC 3780

CAGTTAGAGA CAGATTTACA CACCCCAAAC ACAAAATAGG ATGAACAGGC ACCCAGATGC 3840

AGAGTCCAGG AAATGATGCT GCTTTGGGAT TCAAGAACCC CCTGAGGAAT GTGGAGGAAG 3900

GACACATTTC CTAACAGTAA TTTGAGTATG TGACTCTGTG CGTGACGCTT CTGTGCAGTT 3960

CTGGCGCTAT GGAGAGTGGG TGGACGTGGT TATAGATGAC TGCCTGCCAA CGTACAACAA 4020

TCAACTGGTT TTCACCAAGT CCAACCACCG CAATGAGTTC TGGAGTGCTC TGCTGGAGAA 4080

GGCTTATGCT AAGTAAGCAA CACTTTAGAA TGTGAGGTGG GGCTAGAGGT GAGAAAGTGG 4140

GTTGCAAAAT CCAGCCGAGA CCTCACTCAC AGGAAGAGGC ATGTGCCTCT ATACGTGCAT 4200

ATGTGTGGGC ATGCAAGTCC AACTGTGACC CAAAGTTAGA GATCAGTTCC AGGCAACAAC 4260

AGCTCTAACT AAAAACATTA AATTTAAGAG TAGAAATGAA GATTTGCATA GAAGACCTTT 4320

AGCTTTAGCT CACCATAGCG AGTTCTTTCA TTGCACCTCC ATGGTGGCAT TGCAAGTCTT 4380

GGGATCAGAG CATTGTCCCA GGGTCTCGAT TGGCTCAACC TCATGTGCTT ATAGAAGATT 4440

FIG. 8B/3

TATAAAGACA TGTTGTCTCT CAACTTAAAA GCTCCACCCC AGATGATAAT AATGGATTTT 4500

CAAATTTTGG AACAAGGTCA CTCTGTAATG CAGGCTGGAG TGCAGTGGTG CAGTCACGGA 4560

TCACTGTAGA TTGACCTCCT GGGTTCAAGG TGCTCCTCCC ACCTCAGCCT CCCAAGTAGC 4620

TGGGACTACA TGCGGGCATC ACCATGGCCC TTTTATTTTT GTATTTTTTT GTAGAGCGGG 4680

GTTTTCCCAT GTTGACCCAG ACTGTTCTCG AACTCTTGGG CTCATACAAT CCACCAGCCT 4740

TGCCCTCCCG AAGCGCTGGG ATTGCCGGTG TGAGCCACCA CACCGGCAGC TGCTAATGGC 4800

TTTAATGCAG CCCTTCCTCA ACGTTCAGGA TGTAGTGGAA AGAGCTCTCA GGAAGTGGGG 4860

ATAGCTGGGT TTCAATCCCA GTGCTTCTGG CTCTCTGTGG TCTTGGGTGG GTCACTTAGC 4920

CTCTTGAGCT CAGTTTCTTC ATTATGAAGA AAGGGAATCA TTGTTTCCAT CCCATGAGCT 4980

CATAGGGTTA ATGTGGAATT GATGAAAGAA CATCACAGCA TCCAAGAGGT AAAGTTCTGG 5040

TGGCAGTGGT ACCTGGGTTT TGTTCCCTGG AACTCTGTGA CCCCAAATTG GTCTTCATCC 5100

TCTCTCTAAG GCTCCATGGT TCCTACGAAG CTCTGAAAGG TGGGAACACC ACAGAGGCCA 5160

TGGAGGACTT CACAGGAGGG GTGGCAGAGT TTTTTGAGAT CAGGGATGCT CCTAGTGACA 5220

TGTACAAGAT CATGAAGAAA GCCATCGAGA GAGGCTCCCT CATGGGCTGC TCCATTGATG 5280

TAAGTCTGGG GTGTGGGGCA CAGGGTGGGG AGCTCCAAGT GTCAGGAAGC CTTTTACCCA 5340

ATGAAGGGCA GCATAGAGCT TTTGTGTGGG ACAGAGCGAA TGTTTTGTTT GAGGAAGCAG 5400

GAACTGGCTC TCAACTTTGA GGACTGGGAA TTTCTCAAGG GAGAACAGTT CTTCCGGATT 5460

TTCAATAAAG ACACTGGTCA AGGACATTTC AAGCCCTGGA ATGTCAGTGG AAATCAGTCC 5520

AGAGGCCTGT GTCAGTGGAG GCCTCCCTTG CTGGTGCTCC TCAGTCTCAG CACGCTCCCA 5580

TTAAGCTGGC CACGTACTTG GCTGTGGACC TGAGCCCACC ATTTCCCTAA GAAAGCCTCC 5640

CAGTCACTGG GCTTTCACCA CACCTCCCCG CTTGAGACGT GGGCTTTGTG TTGTTACCTG 5700

GGAGAAGCTA AGCCTGCAGC ACCTTTCAGT GCAAAGAAAT GCTGTGAACT GAGACAGGAG 5760

CCAAGGGTAG GGAGATGGCC GCCCATGGCC AGGCCTCCTT CAGGGGGCAT GCCTTCCCTG 5820

AGGGCTGCTC AGTATATTGA TATGATAATC TTAGTGGTTT CCATTGGGGA GGATGGGGCT 5880

GAAGCTGAAT TCCTGCCCCT TCTTCTCCCA ACACGCCCAA TGGACAGCTT GGAAGGTCAG 5940

TTAGCACACA ACACCATGGA TGAACTTTTT TTCTGTATCA CTTTTCTCCG TCTTTCCTCC 6000

ATTCGTGCTC TGTTGATCTC TCCTCTCTCC CTTTGTCTGT CCCATCTCTT TCTCCTCTCT 6060

FIG. 8B/4

CCTTCCCTTT CCACCCTTCT GTGTTTGTTC TCTCCCTCCC CTGTGTTGTT CCCTACATTC 6120

TCCATCGGGC CTCAGGATGG CACGAACATG ACCTATGGAA CCTCTCCTTC TGGTCTGAAC 6180

ATGGGGGAGT TGATTGCACG GATGGTAAGG AATATGGATA ACTCACTGCT CCAGGACTCA 6240

GACCTCGACC CCAGAGGCTC AGATGAAAGA CCGACCCGGG TGTGTACACC TCCGATTATC 6300

AGAACTGACC ATCCCTCCAA CCCACATGAC CCCGCCCTAT TAGTGTCAGA CTCCCCTCAG 6360

CAGCCAGGGC CTTACCCACA CACCCCCACC TGGCACCTCC CAAGGGTCTG GGTTGAAATA 6420

ACTTGCTCAG CCAAGGCTCC TGAAGAGGGT GCAAGAACCA GGATTTTGGA GGGAATCTCT 6480

GCTGGAGTTT CTGCATATTC CATGGTCCAG GCAGTTCCTC TCATAACGAA CTATCAGACA 6540

GAAATACTTG TAAAGATACT TCATTTATTT TGAAATATTT TTCCTCTTCT AATGTATTCA 6600

TTTATTCATT CAACACTTAT TTTTGAGCTC CTACTATGTT CCAGGCACTC CTCTAGCAAA 6660

CAAAGCAAAT TCTCTCCTCT TTTTCAATAT TTGTGGAAAA AGCAAGGTCT CCCTCTTGTA 6720

GAGTTTATAT TCTAGTATTT TCATAAGTTA TACCTGCTCA CTGGAGAATA CTGAGCCATA 6780

CAGAAAAACA CAGAGGAAAA TTTCACTTAT ATTTTTCCCC ATGTAAAGAT AACCACTCTT 6840

AACATCTAGT ATATGTTCTT CCAGGATTTT TCTATGCACA CACTGAATCT GTATTTTTAT 6900

TTTTAAAATG TTATCATATT GTATGTACCT CTTTGCAGCC TGCTTTTTTC AGTTAGTTTT 6960

TTTGGTTTTT TGGTTTTTTT TTTTTTTTGG AAACCAAGTC TTGCTCTATT CCCTAGGCTG 7020

GAGCACAGTT GTTGCCATCT CGGCTCACTG CAACCTCTGC CTCCAAAGTT AAACTAATTC 7080

TCCTGCCTCA GCCTCCCGAC ATAGCTGGGA TTACAGGCAC ACACCACCAC ACATGGCTAA 7140

TTTTTGTATT TTTTAGTAGA GACGGGGTTT CACCATGTTG GCTGGAATGG TCTTGAACTC 7200

CTGACCTCAA GTGATCCACC TGCCTCAGCC TCCCAAAGTG CTGGGATTAC AAGTGTAAGC 7260

CACCACACCC GGCCTAGTTT GATATTCTTA ATGTGCCCAA AGTATTCTCC TGTAACATTT 7320

TTTAATAGCT ACACAATATT CAAACACACA GATATGTTAT AATTTATTTA CCCAATACCC 7380

TATTATTGGA AAGTTGAGTT CTTTTTTTTC TTTGTTTTGT TTTGTTTTGC TACTATTCTA 7440

AAATGCTATA ACGAACATCC CAATAGATAC ATCTTTGTAT ACATCCATGG TGACTTCCAT 7500

AGGACAGATT CCCAGCAGTA GAATTGCTGG GTTGAATGAT ATGCTTAGGG TAATGACAGA 7560

AGAGTCATTT CAAGCAGCTT CCTAGGGTCT TAGAACTTAA GGATTAATGA GTCTTCCCGC 7620

CCCCTCCCAG TCTATTCAGC ATGATCTGGA TCATGAGGAC TGAGATCTGG AAGAGACTGA 7680

FIG. 8B/5

GATCTGGGAG AGGCTGAGAT ACCAAAAGCC CTGGCTCCAC CCATACCCCT CGCCCTGAAA 7740

ACAGCTCTAG GAATTCCGCG GCCTAGCAAG GCTCCGGGAA GCTCCTTTTA AAGCTGTGAC 7800

GTTAGTAGGC ACATGGACCA TAGAGACCTA TCCAGGGCTC ATGGGACTTT AGTGATCCTG 7860

CCCTTCTCCC AAGGATCCCC CATGGCTGCA ACTTGGAAAT TTCTGCAAAT GGAAGAGCTA 7920

CTCCTTAGGC ACGGTCATGT CTGAGCAGGG ATCTCCTCGG GCTTTCTTAG AATTCTCTCC 7980

CTGGGCACTG GGACTCTTGA TTTCTTGAAT ATTATGTTCC AGGTGGGTGT GGAGGAGGTG 8040

AGGGGATGTA AAGAAGGCTA GACTTGGCCA GGCGCAGTGG CTCATGCCTG TAATCCCAGC 8100

ACTTTGGGAG GCTGAGGCGG GTGGATCACC TGAGGTCAGG AGTTCGAGAC CAGCCTGGCT 8160

AACATGGTGA AACCCCGTTT CTACTAAAAA TACAAAAAAT TAGCTGAGCA TGGTGGCACG 8220

TGCCTGTAAT CCCAGCTACT CGGGAGGCTG AGGCAGGAGT ATCGCTGGAA CACGGGAGGC 8280

AGAGATTGCA GTGACCCGAG ATCGCGCCAC TGCACTCCAG CCTGGGCGAC ACAGCAAGAC 8340

TCTGTCTCAA AAAACAAAAA AGAAAGAAAA AAAGGAAAAG CTAAGACTTA CATGTGTCAC 8400

TTAACCCCTT TTCTCAAACC TCTTTCTCTT CCAGGAATAG TCAACCCCTG GATGGCTTCA 8460

GGGGAAGGGG GATCCTGAAG CCCAGGGCAG CCTCCAACTC TACCCCTTCC TCCTTTGAAG 8520

GATACTAAGG GGTCCAGAAA GGAGGGGCAG GACACTGTTA CCCACCCCAC ATCCCAGCAT 8580

CCACATTGCT CTCTGATGGT CAGGACAGAG CCTTCTCAGG GAGACCAGCC TGTCTGGAGC 8640

TGTGTCTCTT GGCACTCTTA AAGGGCCACT GAAGGTCCGT TCGTGGTCGT GAGGCACACT 8700

TTCAGGGAGC AGAGTGGTCT GTGTCTTCAC AGAGCCCGGA AAATGAACTA GTATGAACTT 8760

TGCCTCCAAG CAGCAGAACT TCTGTTCCCC CGCCCCTAAT GGGTTCTCTG GTTACTGCTC 8820

TACAGACAAT CATTCCGGTT CAGTATGAGA CAAGAATGGC CTGCGGGCTG GTCAGAGGTC 8880

ACGCCTACTC TGTCACGGGG CTGGATGAGG TAAGCCTGGT GGGGCTTGGT GGGGCAAGGG 8940

CACCCTCCTG GGTTAACCTC ATGAAGTCAG GACTTAGCTG TTGGGGCCCC TGCCCTGTCT 9000

GCAGAGCTTG CCTCCAATCA GGACATTCAG TTCAAGGTCC AAGCCACGCC TGGGAGCAGA 9060

GGGGCCTGTG AAACTGGTAG AGGTGGATCC TGCCACAGTT GGTGCACAGT TTATCTTTGC 9120

TTTTCGTGCT AAAGATGGCA ATTTTTCCAA CATTTCCAAT GAACAAATTG AAATATCACT 9180

TAACTTTGCT TTTACAAAGT TGGTTTCATG TGTTCTTGAG CTTCCTGTTC TCTCGTGTTC 9240

AGATAGCTAC AGTTGTCTCT GGGTAGCCAC GGGGACTGGT TCCAGAAGCC CCAACAGTAA 9300

FIG. 8B/6

CAAAATCTGC AGATGCTCAA GTCCCTTCTG TAAAATGGAG TAGTATTTGC ATATAACCTA 9360

TGCACATCCT CCCATATACT TTAAGTCATC TCTGGATTAC TTACGATACC TAACACAATG 9420

GAAATGCTAT GTAAATAGTT ATTGCACTGC ATTGGGTTTT TTTGGTATTA TTTTCTGTTG 9480

TTGTATTATT ATTTTTTCTT TTTTTGAATA TTTTTGATCC ACAATTGGTT ATATGCCAAA 9540

GCCATGGATA CGAGAGGCTG ACTGTTCTGT TTTGCTCCTT CTGGGACTTC TGGGTTTTCC 9600

TGGACCATGT CTGAGACAGG AACGTTGTAA GACCTGTTGC ACACAGTTGG GCAGGTTGTG 9660

CCCTGTACAG AGGGATGGGC TGAGAGGGGC AGTTGCCTGC ATCACCCATT GCAGCAGACT 9720

GGAGGGAGTC TGCTTGTTTG TAGTTCCTCA GTCAGCAGGG GCCTTTTGTC TTTCCTTCCT 9780

TTCCTTTTTT TTTTTTTTTG AGACGGAGTC TCACTCTGTT GCCCAGGCTG GAGTGTAGTG 9840

GCACAGTCTC GGCTCACTGC AATGTCCGCC TCCTGGATTC AAGCGATTTT CCTGCCTCAG 9900

CCTCCTGAGT AGCTGGGATT ACAGGCGCGT GTCACCATGC CCAGCTAATT TTTGTATTTT 9960

TAGTAGAGAT GGGGGTTTCT CCATGTTGAT CAGGCTGGTC TCGAACTCCT GACCTCGTGA 10020

TCCGCCCACC TCGGCCTCTC AAAGTGCTGG GATTACAGGC GTGAGCCACC ACGCCTGGCC 10080

AGCAGGGGCC TTTTTTCTAA TTTATATGAA GACACCTAAT TTATATGTGT TAGCAAAGCC 10140

CTCCTGTTTA TGCCTCACCT CCTCCCCCGA AGCTCATACG GCAGGATGTT CCTGAGAAAA 10200

TTGCCTCTTA GAAGATAGAG AGGAGATGCC AAGCCTAAGT TAGGCAGACT CAGGAGGATA 10260

GGTCTGACCC ACCCCCTGCC ATTCCCCAGC ACACTTGTGA TTAATCTCCT TGGCCAGAGC 10320

CAGGCAGAAC ACCCTCGCGT AAGAGATTTG CCCCCCAGCC CCGTCCCAGC CCTCAGCTAG 10380

ACAGAAGATT CCCTTTCCAG AGAGGCTGCA GAGCATGAGA GCTCTTTCTG TGTGCTTAAG 10440

GTCCCGTTCA AAGGTGAGAA AGTGAAGCTG GTGCGGCTGC GGAATCCGTG GGGCCAGGTG 10500

GAGTGGAACG GTTCTTGGAG TGATAGGTAG GTGAGGGGAC CCCACGGGAT TGGCGGTGGC 10560

GGGGAACAGG GTCCGGGACA AGGCTGTGTT GGGAACTGAG CCATGAGAGT ATTGAAGATG 10620

CTTGGTATAA AATCACCCTC AAAACCAATG ATCCGCAGAG AAGAGGGGCA CAGGTGTTGG 10680

CTCCAGGGAA GGGCCAGGAG TGGAAGCGGG GTGCTGGGGA CCCAGAGAGG TTGCTGACAA 10740

CCATTGGCTG GAAAGGAAGG ATTCCAGAAA GCGTGGGGAA GGTCCAGGCA GGAAAAGCGT 10800

ATGAATGCAG GGTTCTGGGC TAGAGAAGTG ACTTCCCTTC TTGGGGTCTT GTGTTGCCTT 10860

TCCTGTGAAA TGGGAACAGT ATTATTAGCA CTTACCTTGT GGGCTGATAT TGAGGAGTAA 10920

FIG.8B/7

CTGGGACTTG TTTTTGGGCA AGTGCTGAGC CATTGCTAAG ATTCCCCTTA CCCGTGCTTG 10980

TCCCTTGTAT TAAGGCACAA GGGCCCTTTG AAAAGAATTT TACCTGCTTT ATCAATTGAA 11040

AGGGATTAAG ACCTTGGGGG CCAACCCAAA ATAAACATGC GAACTTATTA TTTATAGGCT 11100

CCATGCACAC TTCGTAAAAC CTCCATGGTC CTACTGGTTC CTGATTACCT CCACTCAATG 11160

AGAGGCAATT CATTACTGAA TGAGCCATAA GCGCCTCTTA TTTCGAGAGG GGGATGGCAG 11220

GACTCAGTCG AGGAGAAGGA CCGCACCCAG GCAGCCTGGG CCCCTCGGCT CCTGTACTTA 11280

TTTACTGCTG GGTACTTCCT AGCCCAGCAT GTAATTACTG GTTCGTTCAG TCATTCGTTT 11340

AGTAAATGTT TCTTGGGCAC CTACTACATA GGAGGCACAG GTCAAGGCAC TGGGGATATT 11400

CTTTCTACCC ACCCCCTCCC TCCCTACACT GTGATTAGGG ACTGACCGAT C 11451

FIG. 8B/8

(2) INFORMATION POUR LA SEQ ID NO: 3:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 1834 paires de bases
(B) TYPE: acide nucléique
(C) NOMBRE DE BRINS: double
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 3:

ATTTTTTTTT TTTTTTTTGA GACGGAGTCT CACTCTGCCA CCCAGGCTGG AGTGCAATGG 60

CGCGATCTTG GCTCACTGCA ACCTCCGCCT CCCGGGTTCA AGTGATTCTT CTGCCTTAGC 120

CTCCTGAGTA GCTGAGACTA TAGGTGCCCG CCACCACGCC CAGCTAATTT TTGTATTTTT 180

ATTAGGACGG GGTTTCACCA TATTGGCCAG GCTGGTCTCG AAATCCTGAC CTTGTGATCC 240

GCCCACCTCG GCCTCCCAAA GTGCTGGGAT TACAGGTGTG AGCCATTGCG AGCAGCCCAG 300

AACTCAATTC TTAACCTTTA AAGTATGATG AGAAGAAGGA TCAAGCCCTC ACCAGCCCAT 360

TTAAGGAGTT TAGGCTCAGT CTTGAGGATG TGAGAAGTCA TTGCTATTGG GTTTCACACT 420

GAGGTTAACA GGTGAAGTCA GCATTTTGGT AGTTCACAGC AGCTGCAACT CTTTGTATTT 480

CTCTGATACC TCCTGTCCCA ACCTACATCA GGCCTTCCCT TCTTCCTGCT TCCTTAATTC 540

CTCCATTTTC CCACCAGATG GAAGGACTGG AGCTTTGTGG ACAAAGATGA GAAGGCCCGT 600

CTGCAGCACC AGGTCACTGA GGATGGAGAG TTCTGGTGAG TCCAGAACCC AGGAAGACCC 660

AGAAGGGTAA GGGTGGGGAA GAGAGGGGAA ATCTCAGACC TCAGTCCCCA GCTAAGGTTA 720

TCAGATTCCA GCCCTTGGGA GATCTTGGCT GTGTTCTCCT CCAGCCCAAG GCCCAGCAAG 780

GATGAGGTTC TGAGAGGAGC CTTCCAGGCC ACAGGGACAA TGAGCCCAGG ACCAGGCCAA 840

CATGACATGG CTCTTGCCTC CTGTGTGCCC CTCCGCCACA CACTCTATTC CAGCCACAGG 900

CACCCTGGCC TTAGCACAAT TCTTTTCTGA GCCTAGGAAG CTCCACTTAC CCTGATCTTC 960

CAACGTCAAC CTCACCCTCT CTCAGGTTGT TTCTATTCAG GCTTCAAGTC TCAGCTTAAG 1020

GAGAATTTTC AAGTCTCAGC TTAAGGAGAG CCCCCTAAGT TCCCCGAGGA CTGGGATTAA 1080

TTTATGATGC TCATCACCCT TAAAATTGTT TGCTTAAGCC GGGCGCGGTG GCTCACGCCT 1140

GTAATCCCAG CACTTTGGGA GGCCGAGGTG AACGGATCAC GAGGTCAGGA GATCGAGAAC 1200

FIG. 8C/1

ATCTTGGCTA ACACGGTGAA ACCCTGTCTG TACTAAAAAT ACACAAAAAA AGTAGCCGGG 1260

CGTGGCAGCG TGCGCCTGTA GTCCTAGCTG CTGGGGAGGC TGAGGCAGGA GAATCACTTG 1320

AACCTGGGAG GCAGAGGTTA CAGTGAGCCC AGATTGCGCC ACTGCACTCC AGCCTGGGCG 1380

ACAAGAGAGA CTCTGTCTTG GAAAAAAAAA AAAAAATGTG GTCTTAGTTT AATGTCAAGG 1440

GAAAGGTTTT GGGTGTTTTT ATTACTTTAT TTTTTATTTA AAAACTATAA TAGAGACGGG 1500

CCTCGCTATA TTTCTCGGGC TGGTCTCAAA CTCCTGGGCT CAAGCGGTCC TCCCACCTTG 1560

GCCTCCCAAA ATGCTGGCAT GTGGGCCTGG TCAACATATG GGACCCCAAC TCTACAAAAA 1620

ATTTTAAAAT TAGCCAGATG TGGTGGCGTG TGCCTGTAGT CCCAGCTACT TGGGAGGCTG 1680

AAGCAGGGGG TCACTTGAGC CCAGGAGGTT GAGGCTGCAG TGAACTATGA TTGTCGTTCA 1740

CTTTTCTTCT GAACGTGAGA TTAAGTGTAG TCAGCAATTT GGCTTAGGAT TATTTATTCA 1800

GAATTTTTAA CCGTCACGTT GCGGCAAACC AGGT 1834

FIG. 8C/2

(2) INFORMATION POUR LA SEQ ID NO: 4:

(i) CARACTERISTIQUES DE LA SEQUENCE:
(A) LONGUEUR: 14664 paires de bases
(B) TYPE: acide nucléique
(C) NOMBRE DE BRINS: double
(D) CONFIGURATION: linéaire

(ii) TYPE DE MOLECULE: ADN (génomique)

(xi) DESCRIPTION DE LA SEQUENCE: SEQ ID NO: 4:

AGGAGGTGGA GGTTGCAGTG AGCCAAGATC ATGCCACTGC ACTCTAGCCT GGGCAACAGA 60

GCGAGACTCT GTCTCAAAAA ATACACACAC ACACACACAC ACACACACAC ACACACACAC 120

ACACACATAT ATATACACAC ATATATATAC ACACACATAT ACACACACAC ACGTCTGTAT 180

ATATATGTGT GTGTGTATAT ATACACACAC ACACTATTCT ATATATTCTT GTAGAGCTAT 240

GTGTGTCTCC TGTGCTATTG AGCATGAGCC CTTTTTTTTT TTTTTTTTTT TTGAGACAGA 300

GTCTCACTTT GTCGCCCAGG CTGGCATACA ATGGCGCAAT ATCGGCTCAC TGCAACCTCC 360

GCCTCCTGGG TTCAAGTGAT TCTCCTGCCT CAGCCTCCCA AGTAACTAGG ATTACAAGTG 420

CCCGCCATAA TGCTCAGCTA ATTTTTGTAT TTTCAGTAGA GATGGGGTTT CACCATGTTG 480

GCCAAGCTGG TCTCAAACTC CTAGCCTCAG GTGATCCACC TGCCTCAGCC TCCCAAAGTG 540

CTGGGATTAC AGGCATGAGC CACAGCACCC TGGTGAGCAC TAGAGCTTAT TTCTTCTATC 600

TAACTGTATT TTTGTATCCA TTAGCCACCC TCTTTTCATC CTCCCCTCTC CTTCCCTTCC 660

CAGCCTCTGG TAACCACTGT CTGCTCTCTA CTTCCATGAC ATATGCTTTG TTTTAGCTCT 720

CACATATGAG TGAGAGCATG CGACATTTAT CTTTCTGGCC CTGGCACATT TTTGAATCAT 780

TGTTAGAAAA GATGATGGTT TGGAGTAGAT ACATCAGAAG TGACAGCGTT TGCCCTAAAA 840

AGGAAAGACA GGCTCCTCTG GGACCCTGAC CAAGTTCCTG TGAACTATTT TATTATTGTG 900

CTGTGTTAGT CCTGGGGTCT TCCGTTCCCA GCCCTCCTCA CCTGCTCCCA TATGGCTCTC 960

TCTCTTCTTC CAACCTCTCA GGATGTCCTA TGAGGATTTC ATCTACCATT TCACAAAGTT 1020

GGAGATCTGC AACCTCACGG CCGATGCTCT GCAGTCTGAC AAGCTTCAGA CCTGGACAGT 1080

GTCTGTGAAC GAGGGCCGCT GGGTACGGGG TTGCTCTGCC GGAGGCTGCC GCAACTTCCC 1140

AGGTGGGAGA TGCTCTTGAT GGGGGGAGGG TCTAAGCCGA AAAAGTTCCA GGCAGAAGAA 1200

FIG. 8D/1

GCCTAACTAG TGCTTATTAA GTCTCTCTGT TCCAGACGTC CACTATCTTA TTAAACCTTC 1260

CCTGTTTTAC TGAGAAGGAA ACCACCATGC TGAGAAGTTT GCAATAGGGA GCTGGGTAGC 1320

AACTTTGGAA GCAGGAACTT GTGGGAACAA TGCAGATGCT GCTTGGACTT ACGATGAGGT 1380

TATGTCCAGA TAAGCCCATC CATCTTTTGA AAATACCCTA AGTGAAAAGT GCATCCAATA 1440

TGCCTAACCC CCCAAACCTC ATAGCTTACC CTGGCCTACC CTCAAACATT GCTCGGAACC 1500

CTTGACCTTA AGCCTAAAGT TGGGCCAAAT CATCTAACTC CAAAGCCTAT TTTACAAAGA 1560

AAGTTGTTGT AATATCTCCA TGTAACTTAC TTAATACTTG TACCTAAAAA GTGAAAAACA 1620

AGAATGGTTG TACGGGTACT CGAAATCCAG TTTCTACTGA ATGTGCATCT CTTTCACATT 1680

GTAAAGTTAA AAAATTGTAG CCGAACCATC CTAAGTCAGG GACTGTGAGT ACTGTGTCAG 1740

TAACAGTAAG GGCACTATTG GAGAACCAAG TTAGCAGCTG CTGCAATAGT TCAAGTCAGA 1800

GATGATGAAA ACCTAGACCA AGTCAGTAGC AGCAGAGATG GAGGGGAGAC AGCAGATTTA 1860

GGGAGAGCAT ATTGGGTGAT GTAGGGAAGG AAGAAGAATG ATGTCAAGAT TCCCAGTTGG 1920

GGACCTGACA ACATTGCAAC ATAAGACACA CAAGAAGATC GGGTGGGTGG CTCATGCCTA 1980

TAATCCCAGC ACTTTGGGAG GCAGAGCCAG GAGGATCACT TGAGCCCAGG AGTTCAAGAC 2040

CAGCACAGGC AACATAGTGA CACCTCATCG TTACCCAAAA TAAAAAAAAA AATGAGGTGG 2100

GAGGATTGCT TGAGCTCGGG AGGTTGAGGC TACAATAAAC TGTGATCATG CCACTGCACT 2160

CCTGCCTGGG TGACAGAGTG AGACCCTGCC TCAAAAAAAA AAGACACACA AGAGAAAAAT 2220

ATCAGCGTGT TGTTTGTTTT TGGTGGAGTT AATTGTGGGG TTCTAGGGAA AGGAATTTAG 2280

CTTGGGACAT GGAAAGTTTG AGGTTCCTGT AGAGTGTCCC AGTGAAGATT TGTAATAGAG 2340

CATCGGATGC GCATATTAGA TGGCACTTGG TGATATGATA AGAACTCAAA AAATATTTGA 2400

GGAATAAAGG AAAGAAGAGG CCAGACGTGG TGGCTTATGC CTGTAATCCC AGCACTTTGG 2460

GAGGCTGAGG CAGGCGGATC ACTTGTGGTC AGGAGTTCGA GACCAGCTTG GCTAACATGG 2520

TGAAAACCCA TCTCTACTAA AGATACAAAA ATTAACCGGG GATGATGGTG GGTGCCTGTA 2580

ATCCCAGCTA CTTGGGAGGC TCAGTCAGAA GAATCGCTTG AACCCAGGAG GCGGAGGCTG 2640

CAGTGAGCCG AGATCGCGCC ACTGCACTCT AGCCTGGGCA ACAGAGCCAG ACTCCGTCTC 2700

AAAAAAAAAA AAGTGAGAGA GATTGAGGCT GGGATATATG GCTCAGGCAT CATGCGCGTG 2760

TAGGGGGCAG TTAAAAAGCA GAAGTAAGAA AGATTGCCTA GGGAGGCAGG AAGGGTGAGG 2820

FIG. 8D/2

TGAGAGGAGA AGAGGCCCAG GACCAGATTC TAGTCACCAA CAGCGTTTAA GGGGCAGGTA 2880

AGGAAAACAA AACCATCAGC AAAGACTGAG AATGAAAGCC CAGAGAGGAA GGAAAAGCCA 2940

CACATACAAT CAGTACAGCT CCATCTGAAT AAAGGTAGCG CCCCCCCCCC CCCAAATCAT 3000

TAGAGAAATG CCTGATTCGG TTTTCTGTGG ATTTTTCCTA AGAACCTAGA TGTGGGGAAT 3060

AGAAATAAAT GGTTCCCTCT GTCTCATCCC CTCCCTGCCC TCTGAGAGGA AGCTGTGATT 3120

GCGTGCTCCC TTTCTGGGGG TGCAGATACT TTCTGGACCA ACCCTCAGTA CCGTCCGAAG 3180

CTCCTGGAGG AGGACGATGA CCCTGATGAC TCGGAGGTGA TTTGCAGCTT CCTGGTGGCC 3240

CTGATGCAGA AGAACCGGCG GAAGGACCGG AAGCTAGGGG CCAGTCTCTT CACCATTGCC 3300

TTCGCCATCT ACGAGGTGTG TAGTCCTGAT TGGCTCCAGC CCAGGAAACA TACTTTCCCA 3360

GAGAGGACGC TTCCAGGGGC TTCTAGAGGG GCCCTCTGCT TCCTCAATAC CAGTGACCCA 3420

CAGAGCTCCT GGTATCAGGA CCACTTGTGT TTGTAACAAG CAAAAAATAC CAGGGGGGGC 3480

ATTAGAGAGG CAGTGGAGCG GGCCTGGCAG AACAGGTGCC TGGGGGTCAG GCTTCCGCAT 3540

GCGGGCTGCA GTTGCTGGCA TTGCCTTCCG CAGGCTCCTC ATCCTCATTC ACATCTGAAG 3600

CATCTTCCTT TCTGTTTCTT CTCAAGGTTC CCAAAGAGGT ATAGCAGCAG CAGCGGCCAG 3660

CAGTTGTGTG CAGCACTACC CAGGGGGGCC CGAGTCTGTC TGTGGCTCGT CGAGAAGCTT 3720

CCTGGTGGGG TTTGTGGGCA GGACTTGTGA TAGGAGAGGG CCTTGCCTGT TGTTATTTCC 3780

CACTTGCAGA GCAGGTTGCC TCAGGGCATT GCATGACCCA TGACTACCAC CCCCAGGATG 3840

TGCACTTTCT CCCTCGCACC AGACACTGCA CGTCACACAC ATGCCTTTGC ACACTCACCC 3900

TCCTCCACGC TTACAGCCAC ACACACAGTC ACACAGACGC GTTCTGAGGG TGGCTGCCCG 3960

CTTGGGATGG AGGAATCACT TCCCTCAGAA CCCAGCCAAG TCCTCTAGGC CTCCTTGGGG 4020

GTCCTTCCAG CCTGAGGGGC TTCGGAGCTG AGGACAGCTG TTCTGGTAAG TGTCCCTGAG 4080

TGTGGGGATG ACACATTTCC ATTCACTCTG AATCACAACA GAAAAGGGAA GAGGAATTGA 4140

GGTAGGGAGC CTATTTAACC CTTGGGAGTC GGGAAGTAGG GAGGTTGAAA CTGTGACATG 4200

GGTGACCAGG GAGTTGGGAA GGGACCCTTG GAGGTGGCTG TGGCAGGACA GGACGTTCCT 4260

CCCGAGGGGC TCATGTGCCC TGGGCTCTCC CCATCTCTCA GATGCACGGG AACAAGCAGC 4320

ACCTGCAGAA GGACTTCTTC CTGTACAACG CCTCCAAGGC CAGGAGCAAA ACCTACATCA 4380

ACATGCGGGA GGTGTCCCAG CGCTTCCGCC TGCCTCCCAG CGAGTACGTC ATCGTGCCCT 4440

FIG. 8D/3

CCACCTACGA GCCCCACCAG GAGGGGGAAT TCATCCTCCG GGTCTTCTCT GAAAAGAGGA 4500

ACCTCTCTGA GTGAGTGCTG GCCCAGCTTT CCCACGTGTT TCTAAAAGCT CACATGGCCC 4560

ACTCCAGAGG TTGAAGGCAT GAGGCAGCTA GACACGTCTC CTCCAGGGTC CTTCTGCTGC 4620

TCCTGAGCCA CTGGCCACAT TACCCCCATT CATTCATTCA TCCATTCTGT GATATTTATT 4680

GAGCACCTAC TATGTTCCAG GCACTGTCCT AGGCACTAAG GATAGAGTAG TGAAGTAAAC 4740

AGAAAGAAAT CCCTGCCTTC ATGGAGCTTA ATATTCTAAC ATGAGACAAT AATGGATAGG 4800

AAAAACATAT GTAGCATGTT AGATTTGGAG AGGTGATATG GAGCAAAAAT AAAGTAGGGA 4860

AGAGGGATAG GAGGTGTTGG GGATGCTTGA AATTTTAGGT TAGCATGGCC AGGAAAGCCA 4920

CATCCTGTCC CTGGCCACCA CAGATGAGCT CATAGCCCCT GCCACTCTGA TCTCTGTCCT 4980

TGGAAGATGC ACCAGGTCCA TGGGTAGGTG GCTGGGTCAT GCCTTTGGGG GGCTCTGAGC 5040

AATACTAACA AGAACCTGCG TGCCTGGGCT TGGCTGTCGG GGATGGTGCT GACATGGGGC 5100

TGGTTCCTGG GGTTGGGGTG TTCCAGGGGT TCTCTAGAGG CTGGTTCTGG CTTGGCTGCC 5160

AGGAAGCCGT GCACCAGAGC AAACCGTCCA CGGGCCTCCT GCTTGCTTCT GGTGACACTG 5220

AGACCCCACA TGTCTGTATT CCTCACAGGG AAGTTGAAAA TACCATCTCC GTGGATCGGC 5280

CAGTGGTGAG TGGTTTAGAT CTTCTGTGCG AAAAGTCCAG AGGGTCCCCT TCCCTGACCA 5340

TGCAGGGGAC AGATGGTGCA GGGGAGAATG GGCACTGGCA GAGGGAATGG GAGTCTGGGC 5400

TGTGCTGAGC AGTCCCTCCT TGGCACTGCA AATCCTACTT TGGCATGGCC AGAAGTAATC 5460

GGCCTTAAGC ACCGGGGGCC ATTGAGGCAG TTCAGGGGCT GGGAAATATG GAAGAGGGTC 5520

CTGGAAAGGA GAAGCAATTT GAACAATCGG AGGGAACAAG GCCACAGGAA GGGATGACAA 5580

GAGCCGCAGC GAACACTGGA TTCTGAGACT GGATAACATT GGATTTCACA CATAGAGAAA 5640

AGAAAGTAAG CTGGTGCCGG ACCTGGTGTT GACACTTGGA TCCTCCACTT ACCAGCGGGG 5700

TGACCTGGAC AATTTCTGTA ATCCCTCTCA CTCAGTTTCC TACTCAGTAA AACGGGGATG 5760

ATAATGTGCC TTGCAAGGCT TTTGTGAGGC TTCATCAATG AGGTGATGTA TGTGAAGTGT 5820

CTGGCACAGC ATGGGCACTC AAACAGAGGT GCTTTTTCAC ACTTTACACC TTACAAGGTA 5880

CTTTTCACAT GTGTCATCGC GATACTTGCA AGGTTGCTGA GAGGTAGATG GGGTTATAAT 5940

CCCTGGTGTT CAAGAAAGGA AGCAGAGGCT CAATGGGGTT GAATGACTTC TCTGAGTTCA 6000

CAGAGCTCAG TAAGTGGCAG GGTTTGGAAC TCACATTCAG ACTCTCTGAC TCCAGACTTA 6060

FIG. 8D/4

GGTTTTTCCG CACCTCCACG CTGAGGCCAG CCCCAGGCAG TGAGAAGCCC AAAGTCCGAA 6120

GCACAGAGTG CTGTGTGTTG GGCTCTGTGT GTTGAGGAGT CTTGTGACTG CCTTGGGGCT 6180

TTGGGCTGTA GTCAGCTGAC AGTCCTTTGT GCTCTGTGGG GATGACGTAG GCCAATGGGA 6240

GGACAAATGC CCCTCTGAAC TGTCTTCTGG GCAGTGACAG TCATGGTCAT AATCCTGACC 6300

CTGAGCCAGT GCCAGGTCTC CAAGTGCCTT CTGAATGACC ACAGGCGATT GGTTTTAGTG 6360

GTAGGTGCGT GGGGATCTGT TCTGGTCATC TGGATGCTGG TCATCGGGTG CAGTATTGAT 6420

CAGGACCTGC AAACCCAAAA GCTTATGGGA GCTGGCACGT CACGTGAGTA GAGCAGGCAG 6480

GTGCAGGGTT TTTGATGTCC CTGCACTGAC ACAGTTGTCT GCAGTTCTCC AATTTGACAT 6540

TTGGGCTCCA GTGTCGAGGG TCAAACAAGG AATTTTGGGG CGTGGGCCAA ATCTGGGAAG 6600

ACACAGGGAG CAGGGCCCTT TGGCTCAAGC TGATAGTTGC CGCAGGGATT ACCAGGCCCA 6660

GGGCAGCCTG CCACAAGCTG GGGCTTTTAC CAAAGAAAAT CTCCCTATGT TAAATGCTTG 6720

CTCAAAAATT TTTAAAAAAT ATTCTGTAAG TCAAAATCCA TTGTTAGGTC AGTTTGAGAG 6780

AGCCATGTTT TTGGTGTTTT AGTAACCAAT TTCATTTTTT TATTATTTAT TTATTTGTTT 6840

ATTTTTGAGA CGGAGTTTCA CTCTTGTCAC CCAGGCTGGA GTGCAATGGC ATGATCTCAG 6900

CTCACTGCAA CCTCCGCCTC CCGGGTTCAA GCAATTCTCC TGCCTCAGCC TCCTGAGTAG 6960

CTGAGATTAC AGGTGCCCAC CATCACGCCT GGATAATTTT TGTATTTTTT AGTCGAGATG 7020

GGGTTTCACC ATGTTGGCCA GGATAGTCCT GAACTACTGA CCTCAGATAA TCCGCCCACC 7080

TCAGCCTCCC AAAGTGCTGG GATTACAGGC ATGAGCCAGC ACGCCCGGCC ACCAATTTCA 7140

TTTTTTAAAA AAGGAAGAAA GAAAACCTTA GCCAGAAGAT CTTTTTCCTT GCCATATGCA 7200

GTAAGAGTAG ATTATAAAAA CAAAGTCAGA GCAGTCACTG GTGTCTGGGC ATGGAGGAGA 7260

AAGAAGAATT CTCTTCTCCC TTCACCCTCC ATGCCCCTTT TTGGCTCCAT GTGATTCAGA 7320

TTTCTGGACC CTGGAGCCCC ACCCCAAGCT AAAGACCAGG ATACAGGGAA GCCACAACCA 7380

CTGGCGGTTC TGAGAACTTA CTTTTCACTT ATTCTGCATT TACTGTTTCC TTTTCTTATG 7440

CAGAAAAAGA AAAAAACCAA GGTAGGTGTG TGGGTAGAGA GCATGAAGTG TGTGTACTCA 7500

TGCATATGTA TGTGCATGCA TGTGAAGTGT GCATGTGTGA GCTCATATGC ATCCATGCAC 7560

CAGACTTGCC TCTTCCTCCC CCTCCTTCCT GAGCTTCTGC TGGGGCCGAG CGTGCAGTAA 7620

TGACAACTAC GATTTGCTGG GGGAAGGCTA CGTGCCAAGC ACTCTTTTAG GTGCTTTCCA 7680

FIG. 8D/5

TGATTAATTC CTTCCTCACA ACAGCCCTAT GAGATTAGTA CTATAACTAT CCCCATTTTC 7740

AGAGGGAGAA AAGGTACAGA CTTGACTAAC TTGCCCAAGG CCACACAGCC AGAGAGGGGC 7800

AGAGCCAGTA CTTAGAGCCA GGCAGTCTGG GTCCAGAGTC CGTGTCCTGA ACCACAAGAG 7860

GCCATCATAC GCCATCAGAT TTGGTGCTAG CATTTCTGGT GGTGCCTGGT GGTGATGGAT 7920

CCATCACAGG GGTCCTCCAG GTACTGGTGC TGGCCCAGAC CAGAGCTGAC ACTCCTCAGG 7980

CACTACCACA TTCCAGGCAC TGTGCTTGGG GTCAGTCCCT CTCTTTTTTT TCCCCCCCAA 8040

TTATAACAGT ATCTACAAAG TAGGTGCTGT TATTTTTCCC CTTTCACAGG TGAGATAGAC 8100

TCAAAGAAGT GAACTTGCCC AAGGAACAGA ACTAATGAGT GGGGAAAATG GAACTGGAAA 8160

CCATGTCTGT TTACTCCAAA ACCTGTGTTT CTTGCCCTCT TTCTCTGATG CCAGCCCCCT 8220

ACACTTCAAG GCCTGTGTTG TCCAGACCCA CACTCGGGCC TGCCAGTGTG TGCCTGGCAG 8280

GGATGCTCCA TGGCCACACC ATATCCATCC TACACATCCC CCCTCAGACT GTGACCTCCA 8340

TTTGCTCTGG GATCCCCACA AGCTTCAGCT GCTTGAGCAA GACACTGCTT AGAAGGCAGA 8400

GCAAGCCAAG GCCTCTGGGG CCTGCTGGGA GCCAAAGCTG GGGAGCCGTT TCCACGGGTC 8460

TATCTGCTTG AGCTGTCCTA GATGAGCAGC ATGGAAGGGC AGTGGTGCAT GAGTCCAGGC 8520

GGGCTGCTTT TCTGCTCCGA GAGGCTCTGC CTGCCCAGTT GTTCTCTGCA TTGCAGCCTC 8580

AATCCCCACA GCCTTGCCTT CCCCCGGCTT TCCCTACAGG TGCACCGCAT CCACAGTGTT 8640

GGCACCATGC AGCAGCCGCT CTCCGTCCTT TTCATATCCT TGTCACTTGC ACGAGCATGT 8700

CTTGAAAATA TCCCTTGTTT GTGTAGCATC TTAAATGTTT TTGCAGTATG ATTTTGCATT 8760

CAGTATCTCA TTTGATCCCC ACAAGAGCCC TATGAGGAGG GAAAGCAGAT TTTACCATTA 8820

AAGGATGAGT AAACTGAGGC CAGAGAGGAT ATTTTTGGTT TTTTTTGAGA CAGTCTCACT 8880

CTGTCACCCA GCCTGGAGTG CAGTGGCTTG ATCTTGGCTC ACTGCAAGCT CCACCTCCCA 8940

TGTTCACACC ATTTTCCTGC CTCAGCCTCC CAAGTAGCTG GGACTACAGG CACCCACCAC 9000

CACACCCAGC TAATTTTTTT GTATCTTTAG TAGAGATGGG GTTTCACCCA GTTAGCCAGG 9060

ATGGTCTTGA TCTCCTGACC TTGTGATCTG CCTGCTTCGG CCTCCTAAAG TGCTGGGATT 9120

ACAGGCGTGA ACCCCCCTGC CCGGCCAGAG AGGATATTTC TTAATGAGGG GCAGGGCTGG 9180

GATTCCAGCC CAGTGTTCTG ATGGCTCACC CACTGACCAT TCCACTAATC CGTGTCCTTT 9240

TTCAATCTAA ACTTTCAGGG TTGTAGAGGT TCCTTTGAGG TGCCTCAGTA CTTCCATGGT 9300

FIG. 8D/6

GATGTGGGGT CTGAGGGCCA AGAGCTCTGT TCTCATTAAT CAGAGAAGCT TGTGTTTTTA 9360

AAAACACCAT GTTTACTGCA GGAAATTTAA TTGGACAGTG TTTCCATCTG GAAAAAAAAA 9420

AGTCTACAAA ATACTTGACA ATCACTGCAC TAGATCATGC TGCTTTTAGC ATTCTTAGCA 9480

TTTCACGTGC TGAGCTCTCA ATACTCTACC ATGAGGAGGG ATGGAGTGGG TATGAAAAGA 9540

TAAAGAACTG AAGTCACACG GCTTGTCAGT GGCAGAGATA GAGCTTGAAC CGAGGTTGAA 9600

GAGCTCCCGC CTATTCCTTT CCTCTTCTCA CTGGATAAAG CTGCTCCAAG AGAGGTGCTG 9660

CCTCAGTGTG CCTGTTCAGA CTGTAATCCT CCCTTCCTTC CTGCCTCCTC CCTCCTCTCT 9720

CCAGCCCATC ATCTTCGTTT CGGACAGAGC AAACAGCAAC AAGGAGCTGG GTGTGGACCA 9780

GGAGTCAGAG GAGGGCAAAG GCAAAACAAG CCCTGATAAG CAAAAGCAGT CCCCACAGGT 9840

GTCTGGGCAT GTGGCATGGG TGGGGTGGCC AGCACGCTAC AGGGGCTTCC TATGCGCTTG 9900

GGATACACAG GGGCTGGAGG CTTCCCAGGA GTTTGTCTTG AACATCTGGA GGTTTGAATT 9960

TGTCCCACTG ACCTTTTCTT TCAGCAAGTT CCCCTGAAAT TTGGGCTGCT GCTTGGGTGA 10020

ATATCCCAGG ATGGGGGTTC CATTCTAGGA GTGGACTGGC AGGCTGAGCC TCCCATGGAG 10080

CTGATCCAGC CAGGATACAG AGAAGGGGAG GCAAAGGCTG AGACAGAACC AGCTTGAGAG 10140

CGGAGGCGCA ACTCTTGTCT CCTGGTGGCC TTGAGCATTT CACAATAGGG GGATAAAGGA 10200

TAGGAGCAGA AAAGTGGGGC TGACTTCAGA AATGGGGTCC TCTAGAGCTC ACGGGAGGGT 10260

GTTAGATTGG AGTGGGAGCT TAGTGGAGGT GAGCCTTAGA GGCAAAAGTC TCCAGACCAA 10320

TCCAGGCCCC CTCTTCTATC CGGGGGCCCC TCTTCTATCC AGGGCCCCTC TTCTGTCTGG 10380

GAGCCCCTCT TCTATCTGGG GCCTCATGCA GTGGGGCCTA GGGGAGGTTC TCTGAGGACT 10440

TGGCCTTGAT GACAGGGTGG CTGGAGGAAT CAGAACGGTC AGACCTTCTT TGACCTGCGG 10500

GCACCTTTAG TTGGAATGCT CAGGCCTGGG ATGGTGGAGG GGGCTCTTGC AGGTGGGGAC 10560

TGGGGTGGCG GGGAGGAGGC TGTATGGCCG CCATATCTCC TTTGGCTGGG GGCGTCAGGG 10620

CTGGAGAGGT GTGAAGAGTC CCTGAGGCCT CGATGCATCT CACTCCAGCT CACCAGGTCT 10680

GCATTTGCCC GTCCCCAGCT CCTGCTGCCA CCCCCGGCCG TTTTAGGCAC TTGGCTCCCT 10740

TGGCCCAGAG GAGCTTGCCT CACAGGCCTG TGCACCTCTG ACCCCTGTGA ACCAGTTTTC 10800

CTTTGTGCCT CCACAGCCAC AGCCTGGCAA CTCTGATCAG GAAAGTGAGG AACAGCAACA 10860

ATTCCGGAAC ATTTTCAAGC AGATAGCAGG AGATGTGAGT ACCTCCAAGC CCAGGACGCC 10920

FIG. 8D/7

CACAGGTGCT TCCTTCTCTC CTGGATTAAC TGCTCAGATT ACCAATTATT TCATTATTGT 10980

TTGGTAGAGG TCACTTTGGA CTTCGGTGGA GCCAGGGGAT GTGTGCGTAG CACACAAATC 11040

CACAAGCCCT TGAGTTTTGG ACTGCCACGT CTGCTGGGGG GCTCAGAGGC CTTTTTGCTC 11100

TGAGCTGCCC ACGGTGGTCC TGATAGCTGA GGTGCAGTAT CTGGCCCCCT GTCTTCCTCA 11160

GAAAAGCCCC AGCTTCCCAT GACATAATAG CACCGACAGG GATTTTACAA ACACAGCCAG 11220

GTGGAATTTG TTTTGCAAAG TGTCCGCGCC AGGAGCTGCT GTACTCCTGA ACCATGACCC 11280

TCCTCTCCCT TCCTCCTCAG GACATGGAGA TCTGTGCAGA TGAGCTCAAG AAGGTCCTTA 11340

ACACAGTCGT GAACAAACGT GAGTTGCTCA AACCAAATGG GGGTGGGGTG GGTGGGGAGT 11400

CCCGTTGTCT CAAAGCAGCT CCTCACTCTT CTCCATCCCC CCAGACAAGG ACCTGAAGAC 11460

ACACGGGTTC ACACTGGAGT CCTGCCGTAG CATGATTGCG CTCATGGATG TATCCTTCCT 11520

GCCGCCCCTT CCCGACCCTC TGTCATCAGC CCACGGGGGC CAAGGCAACA TACAGGGTGC 11580

CCAGTCAGGC AAAGGGCCCT AATTTGTGCC CAGGGAAACT TAAGGAGACC CTGATTCAGA 11640

ACATCTTGGA TACTCGTCTG AAAGGGGTTG TTAGAGGCGG AAGGGGAGGA TGTTGGGTTG 11700

TAACTGCCCT AACCCCTGTG CTTCTCTCAG GCCTGGGATC CTGCCCAAGC AAAAGTGGTC 11760

CTTAGGAGAG CGGCTCCTGG GTTACAGAGT AGGCGCAATC TCTGACTGGT GGTGGAGTGG 11820

AGGGGAGGGT TAAATAGTAC AACAGGGCAG TGGGTAGGAC AGCCCGGAGT CTCCTAGACC 11880

CTCCCTCCAA ATCCAGGGGG ATTTTGCTGT GTGCTGTGTA GCCCTGACCT CCCTCCTCCA 11940

GACAGATGGC TCTGGAAAGC TCAACCTGCA GGAGTTCCAC CACCTCTGGA ACAAGATTAA 12000

GGCCTGGCAG GTGGGAAGAG AAAATGAAGC GTGGGAGTCA AGAATGGGGT TGATTTGGAG 12060

ATTCAGTGTG TGACCTCCAT CCTCAAATTT TCTATTGCCA GAAAATTTTC AAACACTATG 12120

ACACAGACCA GTCCGGCACC ATCAACAGCT ACGAGATGCG AAATGCAGTC AACGACGCAG 12180

GTGCTGAGAA GGAAGGGGTG TCAGGGATGT GGACCCGAGA CGGTGGGAGC AGGAATGGGA 12240

GGGGACTAGC TACTAGGGCC CCACTAGAGA AGGAGAGGGA AAGGGCTTCT CACTTTCCCT 12300

TCCCAGGTCA CAGAGTGTCC GAGAGGCAGG GAAAATAGAA GACAGGCCCA AGGCCTCCAG 12360

CTCCACGTCC ACCTCTAACA TGGTCCCCTC CACAGGATTC CACCTCAACA ACCAGCTCTA 12420

TGACATCATT ACCATGCGGT ACGCAGACAA ACACATGAAC ATCGACTTTG ACAGTTTCAT 12480

CTGCTGCTTC GTTAGGCTGG AGGGCATGTT CAGTAAGTGG GAGAGGGGGG CTGCCCTCTG 12540

FIG. 8D/8

CTCTCTTGCA GGGGCAGTTG TGGCAACAGG CATCTCACCT GATAATCTCC AGTCTGCTCC 12600

ATCCAGGCTG AACAAGGGCC AATGACCTCT TTAGGCCCAG AATGGGATGG CAAAGGGAGG 12660

GTTACTGGTG ATTCTCTGCC TGCACATCTT TGTGCTGATG AGGGACAGCA CTGGGCACAC 12720

GGTCCTCTGA GGGGAAGTTA CAGTAGTAGA GGCGGAGTGC GCCTGTAACT GGCCTCTGGC 12780

CTGTGCATTC TTTCACAGGA GCTTCTCATG CATTTGACAA GGATGGAGAT GGTATCATCA 12840

AGCTCAACGT TCTGGAGGTA AAGCATAGGC ACAGCACATT CCCCCTACAC ATTAAAACTC 12900

AAGGTGGAGG GGTCAACGGG GCGGACTGGA CCCAGGGTGT GCTCCTCATT TCCACACAGT 12960

GGTGGAGGGA AGGGATAGGA ACAGAACATG GAGGGAGGCT CAGCAGGCTC CCAGGACACA 13020

TGCACTTGAG GCCCAAAAGG ACCTCTGCTC CCCCAGTCAC TTGATGCGGG AAAACATGCA 13080

CCTTCTTAGG GAAGATCTAG GAGAAAGGAA ACAGTAAGCC ACTGCTTCTT GGAAAATCTT 13140

CTGGGGGTCT GACCTGCTGG GACTGTTCCC TTTCCTCTTG CCCCGTAAGA TTCCTAGGGC 13200

GGGGGGGGGG GGGGGTCACT CTTTTCTGAT CTACATTCTG ATCTTGGGAC TTCTTTCAGT 13260

GGCTGCAGCT CACCATGTAT GCCTGAACCA GGCTGGCCTC ATCCAAAGCC ATGCAGGATC 13320

ACTCAGGATT TCAGTTTCAC CCTCTATTTC CAAAGCCATT TACCTCAAAG GACCCAGCAG 13380

CTACACCCCT ACAGGCTTCC AGGCACCTCA TCAGTCATGT TCCTCCTCCA TTTTACCCCC 13440

TACCCATCCT TGATCGGTCA TGCCTAGCCT GACCCTTTAG TAAAGCAATG AGGTAGGAAG 13500

AACAAACCCT TGTCCCTTTG CCATGTGGAG GAAAGTGCCT GCCTCTGGTC CGAGCCGCCT 13560

CGGTTCTGAA GCGAGTGCTC CTGCTTACCT TGCTCTAGGC TGTCTGCAGA AGCACCTGCC 13620

GGTGGCACTC AGCACCTCCT TGTGCTAGAG CCCTCCATCA CCTTCACGCT GTCCCACCAT 13680

GGGCCAGGAA CCAAACCAGC ACTGGGTTCT ACTGCTGTGG GGTAAACTAA CTCAGTGGAA 13740

TAGGGCTGGT TACTTTGGGC TGTCCAACTC ATAAGTTTGG CTGCATTTTG AAAAAAGCTG 13800

ATCTAAATAA AGGCATGTGT ATGGCTGGTC CCCTTGTGTT TTGTTGTCTC ACATTTAGAT 13860

ATCAGCCATG CATGACTGAA TGGCTTCCAA TCATATACTC ACCTATCACC TACAAGAGAA 13920

CAATGAAAAA CACACACAAA AACAAAATCT TGAATTTTGT AATCATGCCT ATTGCTATTT 13980

CTTGAGCATA AGAATGGCTC AGATACTTTC CAAGACATAA AAGGAAGGCA GAGGAATAGT 14040

TGTTGCTGTA AAAGACATCA AGAATAAATG GGGTCATGTA CAACGGGAGG GGCCGGTTAC 14100

CTGAATAATG GAGTGGAGAT TGAGCTATCC TAGCTCCTCT GCTCACTAAC TGACCTGTCG 14160

CATGACCGTG GACAAAACCC TGAACGCAGC TGTTTGTTTG CTAAACTTCT CTGGACCATG 14220

GCCTGCGGCA TATCTATAGG CATCCTGTGT TTTCCACCCA GTTTCCTTCT TCCTCGCTAA 14280

GCCAACGTGG AAAGGGCTGG CCGTGAATAT GCAGACAAGG TAACGAAAGT AAACCGTCAA 14340

TTAGTAAAAG TACTTCATTT TCCTCTTGTA TTTGCTTCAT TCTTGCTTCA CAAAGTTACG 14400

AAGTCCACAG CTTTATACCA AAATGTAAGA AGGCTATTTG CTTATAAACA TTTTGAGTCA 14460

GGTGTCATCT GATTTCATTC TTCTAATCCA TATTCAATAT TAAAAAATCA GAAACCAAGG 14520

GTGCTGGAGC AGCTCTAGGG CATATATTTC TCTTAAATAG GAGAAAGATT TTCAACAGCT 14580

TTTCCTCCTT GACCCCCTCC TTTCCCAATT TATTTGGGTC ACTACCTTGA ATTTAGAGTG 14640

AATCTGGGAA ATGTAGTCAC CAGG 14664

FIG. 8D/10

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Date of mailing (day/month/year) 23 July 1996 (23.07.96)	
International application No. PCT/EP95/04575	**Applicant's or agent's file reference** B2628A - FL
International filing date (day/month/year) 21 November 1995 (21.11.95)	**Priority date** (day/month/year) 22 November 1994 (22.11.94)
Applicant BECKMANN, Jacques et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

19 June 1996 (19.06.96)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

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